Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibroses and cancer

Julia S. Johansen

This review has been accepted as a thesis together with seven previously published papers, by the University of Copenhagen, December 27, 2005 and defended on May 5, 2006.

Departments of Rheumatology, Hvidovre and Herlev Hospitals, and University of Copenhagen.

Correspondence: C.F. Richsvej 101 B, 2. th., 2000 Frederiksberg, Denmark.

Official Opponents: Steen Gammeltoft and Mikael Rørth.

Dan Med Bull 2006;53:172-209

1. YKL-40

In a search of new bone proteins, the glycoprotein YKL-40 was identified in 1989 to be secreted *in vitro* in large amount by the human osteosarcoma cell line MG63. The protein was named YKL-40 based on its three N-terminal aminoacids Tyrosine (Y), Lysine (K) and Leucine (L) and its molecular mass of 40 kDa (Johansen et al. 1992). This protein was later found to be similar to a protein secreted by differentiated smooth muscle cells from swine explants of the thoracic aorta (Millis et al. 1985), to a protein isolated from the whey protein secretions of bovine mammary secretions during the non-lactating period (Rejman et al. 1988), and to a heparin binding protein secreted by human synovial cells (Nyirkos et al. 1990). In the last few years there has been a growing number of publications concerning YKL-40 and the "Story about YKL-40" has probably just started. The protein has several names: "YKL-40" (Johansen et al. 1992), "Human Cartilage glycoprotein-39 (HC gp39)" (Hakala et al. 1993), "Breast regressing protein 39 Kd (brp-39)" (Morrison et al. 1994), "38-kDa heparin-binding glycoprotein (gp38k)" (Shackelton et al. 1995), "Chitinase-3-like-1 (CHI3L1)" (Rehli et al. 1997), "Chondrex" (Harvey et al. 1998), and "40 kDa mammary gland protein (MGP-40)" (Mohanty et al. 2003). Hopefully, there will in the future be consensus of its name. In this thesis the protein is named YKL-40.

YKL-40 AMINO ACID AND CDNA SEQUENCE

The complete amino acid and cDNA sequence of human YKL-40 was published by Hakala et al. in 1993 (GenBank Accession number: M80927). Human YKL-40 contains a single polypeptide chain of 383 amino acids and has a calculated molecular mass of 40,476 Da (Hakala et al. 1993) and an isoelectric point of about 7.6 (Renkema et al. 1998). The sequence of YKL-40 from several other mammals is known: pig (Shackelton et al. 1995) (84% sequence identity), cow (83%), goat (Mohanty et al. 2003) (83%), sheep (83%), guinea pig (De Ceuninck et al. 1998), rat (80%), and mouse (Morrison et al. 1994) (73% sequence identity). Amino acid sequence analysis reveals that YKL-40 belongs to the glycosyl hydrolase family 18 (Henrissat et al. 1993). This family consists of enzymes and proteins, and includes chitinases from various species (mammalian, bacteria, fungi, nematodes, insects and plants) (Aronson et al. 1997). Human YKL-40 shares significant amino acid sequence identity to bacterial chitinases (Hakala et al. 1993; Johansen et al. 1993 I) (31% sequence identity) and to seven other "mammalian chitinase-like proteins": 1) human oviduct-specific glycoprotein (OGP) (Arias et al. 1994; Buhi 2002) (46% sequence identity); 2) human chitotriosidase (Boot et al. 1995) (52%); 3) human YKL-39 (Hu et al. 1996) (51%); 4) human TSA 1902 (Saito et al. 1999) also named acidic mammalian chitinase (AMCase) (Boot et al. 2001) (51%); 5) mouse YM1 (Jin et al. 1998) (46%) also named eosinophil chemotactic cytokine (ECF-L) (Owhashi et al. 2000); 6) mouse chitinase like protein 2 (45%) (Ward et al. 2001) and 7) mouse protein MGC58999 (43% sequence identity). Three of these proteins have only been described in mouse. All 8 "mammalian chitinase-like proteins" show a high level of sequence identity over certain regions and strict conservation of several structurally important residues including proline and cysteine. The N-terminal amino acid sequence and the catalytic center are highly conserved (>70% identical), whereas the identities are low in the C-terminal sequence. Interestingly, it has also been demonstrated that *Drosophila melanogaster* secretes several proteins, DS47 and imaginal disc growth factors (IDGFs), with sequence identity to YKL-40 (DS47: 34%; IDGFs: 16-23%) (Kirkpatrick et al. 1995; Kawamura et al. 1999). Furthermore, the nematode *Caenorhabditis elegans* and the zebra fish *Danio rerio* have multiple putative YKL-40-like proteins (18%-30% sequence identity).

YKL-40 GENE

In 1997 the human gene encoding YKL-40 was isolated (Rehli et al. 1997). It is assigned to chromosome 1q31-q32 and consists of 10 exons and spans about 8 kilobases of genomic DNA. Recently the transcriptional regulation of YKL-40 during human macrophage differentiation has been described (Rehli et al. 2003). There are probably two independent transcription start sites and the promoter sequence contains binding sites for several known factors and specific binding of nuclear PU.1, Sp1, Sp3, USF, AML-1 and C/EBP proteins. It was further found that the Sp1-family transcription factors seem to have a predominating role in controlling YKL-40 promoter activity. It was also suggested that the YKL-40 gene in monocytes is in an inactive or unstable, yet primed state, which may require additional events (e.g. nucleosome remodeling) that may be initiated by additional elements upstream or downstream of the promoter (Rehli et al. 2003). The genes of the other human "chitinase-like proteins" known so far are also located on chromosome 1. The gene for chitotriosidase (Boot et al. 1998) is located on 1q31-1q32. The genes coding for YKL-39 (GenBank accession number U58514), OGP (Takahashi et al. 2000, GenBank accession numbers U58001-U58010), TSA1902 (Saito et al. 1999), and AMCase (Boot et al. 2001) are located on 1p13. The mouse YM1/ECF-L gene (Chang et al. 2001) is located on mouse chromosome 3, a chromosome that corresponds to human chromosome 1.

YKL-40 STRUCTURE

The crystallographic three-dimensional structures of human YKL-40 (Fusetti et al. 2003; Houston et al. 2003) and goat YKL-40 (Mohanty et al. 2003) display the typical fold of family 18 glycosyl hydrolases (Henrissat et al. 1997). The structure is divided into two globular domains: a big core domain which consists of a $(\beta/\alpha)_8$ domain structure with a triose-phosphate isomerase (TIM) barrel fold, and a small α/β domain composed of five antiparallel β-strands and one α-helix that is inserted in the loop between strand β7 and helix α7 of the TIM barrel. This gives the active site of YKL-40 a groove-like character. YKL-40 is a lectin and bound carbohydrates are not hydrolyzed as discussed in detail below. A 43Å long carbohydrate binding cleft is present at the C-terminal side of the β-strands in the $(\beta/\alpha)_8$ barrel. The crystal structure of YKL-40 is similar in many aspects to the crystal structure of human chitotriosidase (Fusetti et al. 2002), mouse YM1 (Sun et al. 2001), *Drosophila melanogaster* IDGF-2 (Varela et al. 2002) and to other members of the glycosyl hydrolase family 18 (Coulson 1994; Reardon et al. 1995), but several major structural changes are also found. Family 18 chitinases contain a sequence motif DxxDxDxE which lies on strand β4. The glutamic acid (E) is the catalytic acid, which pronates the glycosidic bond. The neighboring aspartic acid (D) plays a key role in orient-

ing the N-acetyl group of the –1 sugar for nucleophilic attack on the anomeric carbon, and stabilizes the subsequently formed oxazolium ion intermediate (Van Aalten et al. 2001). In human YKL-40 there is a mutation of the catalytic glutamic acid to leucine (L, residue 140) and a mutation of the catalytic aspartic acid to alanine (A, residue 138). Both mutations appear to rule out a hydrolase activity for YKL-40. Although YKL-40 is not a chitinase, human YKL-40 binds chitin of different lengths and in a similar fashion as seen in Family 18 chitinases, and nine sugar-binding subsites are found in the 43Å groove (Fusetti et al. 2003). The presence of chitin fragments in the binding groove does not cause drastic conformational changes in the protein (Fusetti et al. 2003). YKL-40 is N-glycosylated at asparagine (Asn, residue 60) and 2 β(1,4)-linked GlcNAc residues are visible in the electron density. Glycosylation is a unique feature of YKL-40 structure as the residue corresponding Asn (residue 60) does not exist in chitinases and is mutated to proline in other "mammalian chitinase-like proteins". YKL-40 binds heparin (Shackelton et al. 1995) and amino acid sequence analysis reveals that YKL-40 contains one heparin-binding motif (GRRDKQH, residue 143-149). This putative heparin-binding site is located in a surface loop (Fusetti et al. 2003). However, soaking of YKL-40 crystals or co-crystallization in the presence of fully sulfated heparin did not result in evidence of binding at this site. It has been suggested that heparan sulfate is a more likely ligand of YKL-40 (Johansen et al. 1997; Fusetti et al. 2003), and unsulfated fragments of heparan sulfate can be accommodated in the binding groove of YKL-40 (Fusetti et al. 2003). Amino acid sequence analysis reveals that YKL-40 contains two potential hyaluronan-binding sites on the external face of the folded protein (Malinda et al. 1999), but this has not been evaluated in crystallization studies. YKL-40 contains five cysteins and four are involved in two disulfide bridges (C^{26}-C^{51} and C^{300}-C^{364}) (Fusetti et al. 2003) and conserved in all "mammalian chitinase-like proteins" (Sun et al. 2001; Fusetti et al. 2002; Mohanty et al. 2003). The free Cxx in YKL-40 is located in a tightly packed hydrophobic pocket. Three conserved *cis* peptides are present.

YKL-40 EXPRESSION IN NON-MALIGNANT CELLS

Several different cell types of ectoderm, mesoderm, and endoderm origin express YKL-40 mRNA and protein *in vitro* and *in vivo* under specific conditions.

Macrophages

YKL-40 mRNA expression *in vitro* is absent in normal human monocytes but strongly induced during the late stages of human macrophage differentiation (Krause et al. 1996; Rehli et al. 1997; Renkema et al. 1998; Rehli et al. 2003) and by treatment of monocytes and the human monocytic cell line THP-1 with phorbol myristate acetate (PMA induces differentiation of monocytes into an adherent macrophage-like cell type) (Kirkpatrick et al. 1997; Rehli et al. 2003). Serial analysis of gene expression (SAGE) demonstrated 288 fold increased YKL-40 transcripts in monocytes stimulated with granulocyte-macrophage colony-stimulating factor (GM-CSF), a 182 fold increase in YKL-40 after stimulation with M-CSF and a 31 fold increase in YKL-40 transcripts in lipopolysaccharide stimulated monocytes (Hashimoto et al. 1999a; Suzuki et al. 2000). No YKL-40 expression was found in human monocytes or dendritic cells (Hashimoto et al. 1999b). *In vivo* YKL-40 mRNA and protein expression are found by a subpopulation of macrophages in different tissues with inflammation and extracellular matrix (ECM) remodeling: 1) macrophages in inflamed synovial membranes from patients with rheumatoid arthritis (RA), osteoarthritis (OA) or ankylosing spondylitis (AS) express YKL-40 mRNA and protein (Kirkpatrick et al. 1997; Baeten et al. 2000; Volck et al. 2001); 2) macrophages in atherosclerotic plaques express YKL-40 mRNA, particularly macrophages that had infiltrated deeper in the lesion, and the highest expression of YKL-40 is found in macrophages in the early lesion of atherosclerosis (Boot et al. 1999); 3) macrophages and giant cells located in the media of arteritic vessels of patients with giant cell arteritis (GCA) express YKL-40 protein (Johansen et al. 1999a, V); 4) giant cells in the sarcoid lesions of patients with pulmonary sarcoidosis (Johansen et al. 2005b) express YKL-40 protein; and 5) peritumoral macrophages in biopsies from small cell lung cancer express YKL-40 mRNA (Junker et al. 2005a). Another "mammalian chitinase-like protein", chitotriosidase, is also produced by activated macrophages but not by the same sub-population as YKL-40 (Boot et al. 1999).

A *Drosophila melanogaster* cell line exhibiting macrophage-like properties secretes a closely related protein to YKL-40 named DS47 (Kirkpatrick et al. 1995). This protein is expressed during the entire *Drosophila melanogaster* life cycle. In the larvae the DS47 message is found in the fat body (an organ that is somewhat analogous to the human liver) and by hemocytes and is secreted into the hemolymph. The IDGFs (proteins with amino acid sequence identity to YKL-40) are secreted by *Drosophila* yolk cells, the fat body and the imaginal disc (cells with macrophage-like properties) (Kawamura et al. 1999).

Using flow cytometry Baeten et al. (2000) showed that RA patients have YKL-40 positive (+) peripheral blood mononuclear cells (PBMC) and that these cells are CD16+ and have a dim expression of CD14. The CD14+,CD16+ monocyte phenotype can differentiate from classic CD14++ monocytes by maturation *in vitro* and resembles the monocyte population described by Ziegler-Heitbrock (1996), but their physiological role remains to be determined. The CD14+,CD16+ monocytes are increased in numbers in patients with RA (Baeten et al. 2000), sepsis (Fingerle et al. 1993), tuberculosis (Vanham et al. 1996) and solid tumors (Saleh et al. 1995). These monocytes are believed to be a more mature version of monocytes with properties of tissue macrophages, probably of proinflammatory type. They have a similar antigen-presenting potential as macrophages and produce proinflammatory cytokines, but produce little or no anti-inflammatory cytokines. They have a low capacity for phagocytosis and reactive oxygen production, and a high expression of major histocompatibility complex (MHC) class II antigens and adhesion molecules (Thieblemont et al. 1995; Frankenberger et al. 1996; Ziegler-Heitbrock et al. 1996).

The present studies show that YKL-40 is a phylogenetically highly conserved protein secreted by macrophages, and the expression of YKL-40 seems to be restricted to small, unique groups of macrophages exemplifying the phenotypic variation among macrophages.

Neutrophil granulocytes

Neutrophil granulocytes share a common progenitor cell with macrophages and neutrophil precursors begin to synthesize YKL-40 at the myelocyte-metamyelocyte stage (Volck et al. 1998). YKL-40 is stored in the specific granules of neutrophils and released after full activation of the neutrophils (Volck et al. 1998; Boussac et al. 2000). Chitotriosidase and YM1 are also neutrophil granule proteins but their exact subcellular localizations are unknown (Boussac et al. 2000; Harbord et al. 2002).

Chondrocytes

Hakala et al. reported in 1993 that YKL-40 mRNA expression is high in cartilage from RA patients and undetectable in normal cartilage. Cartilage explant- or monolayer chondrocyte cultures isolated from RA cartilage (Hakala et al. 1993) and OA cartilage (Johansen et al. 2001c, VII) secrete YKL-40 *in vitro.* Monolayer cultures of chondrocytes freshly isolated from normal cartilage secrete low levels of YKL-40, but this basal production of YKL-40 increase more than 300 fold in first- and second-passage of chondrocyte cultures (Johansen et al. 2001c,VII). Microarray cDNA analysis have demonstrated that YKL-40 gene expression is up-regulated in dedifferentiated human fetal chondrocytes compared to chondrocytes maintained in a differentiated state (Stokes et al. 2002). Chondrocytes cultured in monolayer become dedifferentiated, acquiring a fibroblast-like appearance and changing their pattern of gene expression

from one that express chondrocyte-specific genes to one that resembles a fibroblastic or chondroprogenitor-like pattern. *In vitro* redifferentiation of dedifferentiated chondrocytes investigated by cDNA analysis show increased YKL-40 expression as does *in vitro* chondrogenesis (Imabayashi et al. 2003), indicating that YKL-40 is a differentiation marker in chondrocytes. *In-situ* hybridization analysis have shown that YKL-40 mRNA is undetectable in chondrocytes from normal articular cartilage but is expressed in moderate to high levels in chondrocytes located in the superficial zone of articular cartilage with mild OA. In advanced OA chondrocytes located in both the superficial, middle and deep layer express YKL-40 and this increase with the extent of tissue damage (Connor et al. 2000). High YKL-40 mRNA expression is also found in chondrocytes in the presecondary ossification center of developing fetal cartilage whereas chondrocytes located in the growth plate and mineralized cartilage have lower YKL-40 expression (Connor et al. 2000). Immunohistochemical analysis have shown YKL-40 protein expression in chondrocytes located in both the superficial and middle layer of cartilage biopsies from RA and OA patients (Volck et al. 1999, 2001; Johansen et al. 2001c, VII; Kawasaki et al. 2001). The intracellular presence of YKL-40 in chondrocytes is shown in the Golgi apparatus and the endoplasmic reticulum (Johansen et al. 2001c, VII). YKL-39, another "mammalian chitinase-like protein", is also secreted *in vitro* by monolayer cultures of human chondrocytes isolated from normal cartilage but is a less abundant protein compared to YKL-40 (Hu et al. 1996).

Fibroblast-like synovial cells (synoviocytes)

Synovial cells obtained from the synovial membrane of RA patients at time of joint replacement secrete YKL-40 *in vitro* (Nyirkos et al. 1990), and YKL-40 mRNA expression is found in inflammed synovial membrane from RA patients but not in non-inflamed synovial membrane (Hakala et al. 1993). Dasuri et al. (2004) studied proteins of fibroblast-like synovial cells from RA patients, using two-dimensional polyacrylamide gel electrophoresis and matrix-assisted laser desorption ionization mass spectrometry, and found that YKL-40 is a major cellular protein in these cells.

Bone cells

Monolayer cultures of osteoblasts from adult and fetal bone do not secrete YKL-40 *in vitro* (Johansen et al. 1992). However, YKL-40 mRNA expression is found in end-stage osteoblasts in osteophytic tissue and in primary osteocytes and osteoblasts at sites of endochondral and intramembranous bone formation. YKL-40 mRNA expression is low to moderate in osteoid-forming and proliferating osteoblasts and undetectable in fully mature osteocytes and osteoclasts, indicating a maturation stage-dependent expression of YKL-40 in osteoblasts and osteoclasts (Connor et al. 2000). It is not known if activated osteoclasts express YKL-40.

Vascular smooth muscle cells

YKL-40 is synthesized *in vitro* by vascular smooth muscle cells isolated from explants of swine thoracic aorta during the time of transition from a proliferating monolayer culture to a non-proliferating differentiated multilayer culture (Millis et al. 1985; Millis et al. 1986). YKL-40 secretion continues as the cells reorganize and form multicellular nodules in which cells reexpress markers of differentiated vascular smooth muscle cells (Millis et al. 1985; Shackelton et al. 1995; Malinda et al. 1999). This *in vitro* nodule forming process mimics some of the characteristics of the *in vivo* changes that occur in vascular wall smooth muscle cells following injury where media smooth muscle cells dedifferentiate, migrate, and contribute to the process of restenosis and neointima formation (Schwartz 1997). Immunohistochemical analysis show YKL-40 protein expression in human smooth muscle cells in adventitial vessels (Johansen et al. 1999a, V) and atherosclerotic plaques (Nishikawa et al. 2003).

Liver cells

Hakala et al. reported in 1993 that a strong YKL-40 mRNA expression was found in human liver. However, this could not be reproduced by Hu et al. (1996). The liver tissue used in the study by Hakala et al. (1993) may have originated from a fibrotic liver. Immunohistochemical studies of liver biopsies have shown YKL-40 protein expression in areas of the liver with fibrosis and no expression in hepatocytes (Johansen et al. 1997; Johansen et al. 2000a, VI). Suppression subtractive hybridization analysis and RT-PCR have found that YKL-40 is one of the most overexpressed proteins in cirrhotic liver tissue caused by hepatitis C virus (HCV) (Shackel et al. 2003). The hepatic stellate cell (HSC), the principal effector cell in liver fibrogenesis (Friedman 2000), express YKL-40 mRNA *in vitro* but YKL-40 protein in conditioned media from human HSC have not yet been detected (E. Efsen, manuscript in preparation).

Mammary epithelial cells

YKL-40 in mice is called the "breast regression protein (Brp-39)" (Morrison et al. 1994) because it is induced in mammary epithelial cells a few days after weaning. YKL-40 is not detectable in milk during lactation but is isolated from the whey protein secretions of bovine mammary secretions during the nonlactating period after weaning (Rejman et al. 1988) and in bovine colostrum (Yamada et al. 2002).

Malignant cells

Se Chapter 5.

Other cells/tissues

cDNA microarray analysis have demonstrated that 1) hippocampus tissue from patients with schizophrenia have elevated YKL-40 expression compared to control hippocampus tissue (Chung et al. 2003); 2) *Helicobacter*-infected murine stomachs have increased YKL-40 expression compared to uninfected stomachs (Mueller et al. 2003); 3) YKL-40 expression in ovariectomiced murine chorioretinal tissue is downregulated by 17-β-estradiol (Rakic et al. 2003); and 4) YKL-40 is expressed in normal human neural retina and retinal pigment epithelium-choroid complex, and upregulated in pathological human exudative age-related macular degeneration and experimental murine choroidal neovascular membranes (Sharon et al. 2002; Rakic et al. 2003). Suppression subtractive hybridization analysis of genes from human mesothelial cells, obtained from benign effusions, that differentiate into a fibroblastic morphology show more than 20 fold overexpression of YKL-40 (Sun et al. 2004).

BIOLOGIC ACTIVITIES OF YKL-40

YKL-40 is a secreted protein suggesting that its sites of actions are most likely to be extracellular. Specific cell-surface or soluble receptors for YKL-40 have not yet been identified. The biological function of YKL-40 is not yet clear, but several possible functions have been proposed:

Growth properties

In vitro studies have shown that YKL-40 in physiological concentrations increases proliferation of guinea pig chondrocytes, rabbit chondrocytes and synovial cells, and that YKL-40 increases proteoglycan synthesis of guinea pig and rabbit chondrocytes (De Ceuninck et al. 2001a). Recklies et al. (2002) found that YKL-40 increases growth rates of three fibroblastic cell lines derived from human osteoarthritic synovium, fetal lung and adult skin. The magnitude of the response of YKL-40 stimulation on synovial cells and skin fibroblasts on incorporation of [^{3}H]thymidine into cellular DNA is similar to that elicited by the insulin-like growth factor-1 (IGF-1), and YKL-40 and IGF-1 work synergistically in stimulating growth of the fibroblasts. YKL-40 initiates mitogen-activated protein (MAP) kinase and PI-3K signaling cascades in fibroblasts lead-

ing to phosphorylation of both the extracellular signal-regulated kinase (ERK)-1/2 MAP kinase and protein kinase B (AKT)-mediated signaling cascades (Recklies et al. 2002), which are associated with the control of mitogenesis. This suggests a role of YKL-40 as an anti-apoptotic protein. The PI-3K pathway, and in particular the phosphorylation of AKT, is strongly associated with cell survival (Bakkenist et al. 2004; Downward 2004; Mitsiades et al. 2004). Identity of cellular receptors mediating the biological effects of YKL-40 are currently not known, but the activation of cytoplasmic signal-transduction pathways suggests that YKL-40 interacts with one or several signaling components on the plasma membrane. Recently, Ling et al. (2004) showed that stimulation of human articular chondrocytes or skin fibroblasts with interleukin 1 (IL-1) or tumor necrosis factor alfa (TNFα) in the presence of YKL-40 results in reduction of both p38 and SAPK/JNK phosphorylation, and that YKL-40 suppresses the cytokine-induced secretion of metalloproteinase (MMP)-1, MMP-3 and MMP-13 and the chemokine IL-8. The suppressive effect of YKL-40 is dependent on kinase activity, and treatment of articular chondrocytes and skin fibroblasts with YKL-40 results in AKT-mediated serine/threonine phospholyration of the apoptosis signal-regulator kinase, ASK1. It was suggested that YKL-40 elicits an anti-catabolic effect preserving ECM during tissue remodeling/destruction (Ling et al. 2004).

YKL-40 in mice is called the "breast regression protein (Brp-39)" (Morrison et al. 1994) because it is induced in mammary epithelial cells a few days after weaning. Mammary involution involves programmed cell death, and it has been suggested that YKL-40 utilizes a chitin oligosaccharide binding ability while participating in the various signal transduction pathways that lead to apoptosis of the regressing cells. Mohanty et al. (2003) hypothesized that YKL-40 is a protective signaling factor that determines which cells are to survive the drastic tissue remodeling that occurs during involution.

YKL-40 acts as a chemoattractant for human umbilical vein endothelial cells and stimulates migration of these cells at a level comparable to that achieved with the endothelial cell chemoattractant basic fibroblast growth factor (bFGF) (Malinda et al. 1999). YKL-40 modulates vascular endothelial cell morphology by promoting the formation of branching tubules, indicating that YKL-40 may function in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells (Malinda et al. 1999). Furthermore, YKL-40 promotes vascular smooth muscle cell attachment, spreading and migration, suggesting that YKL-40 has a role in the process of atherosclerotic plaque formation where smooth muscle cells are induced to migrate through the intima in response to exogenous signals (Nishikawa et al. 2003).

In contrast to many other cell types, chondrocytes and the human osteosarcoma cell line MG63 can be maintained in unsupplemented culture medium for about 14 days without any loss of viability (Johansen et al. 1992; Johansen et al. 2001c, VII). Furthermore, the persistence of YKL-40 secretion from these cells and swine vascular smooth muscle cells (Millis et al. 1985) is not dependent on the presence of serum. The lack of a requirement for growth factor supplementation may be due to their own production of large amounts of YKL-40 when cultured in serum free media, indicating that YKL-40 may play a role in cell growth and survival.

YKL-40 may also has a functional role in embryonic development. In the developing mouse heart expression of YKL-40 coincides with morphological changes involving cell migration, altered cell adhesion and remodeling suggesting a role for YKL-40 in cardiac morphogenesis consistent with its established activities *in vitro* of promoting cell migration and adhesion (Nishikawa et al. 2003).

Several other "mammalian chitinase-like proteins", human AMCase/rat iSBLP[58], human ECF-L/mouseYm1, *Drosophila* IDGFs, also have growth factor activity *in vitro* (Guoping et al. 1997; Kawamura et al. 1999; Owhashi et al. 2000), whereas human chitotriosidase has no mitogenic effect on skin, fetal lung or synovial fibroblasts (Recklies et al. 2002). It has been suggested that *Drosophila* IDGFs may have evolved from chitinases to acquire new functions as growth factors, interacting with cell surface glycoproteins implicated in growth-promoting processes such as the *Drosophila* insulin receptor (Varela et al. 2002).

It has been suggested that YKL-40 may be a differentiation marker since elevated YKL-40 expression is found when monocytes differentiate to macrophages (Krause et al. 1996; Rehli et al. 1997; Renkema et al. 1998; Rehli et al. 2003), when mesothelial cells differentiate into a fibroblast like morphology (Sun et al. 2004), when vascular smooth muscle cells differentiate (Millis et al. 1985; Shackelton et al. 1995; Malinda et al. 1999), and when chondrocytes differentiate to fibroblast like cells or re-differentiate to chondrocytes (Stokes et al. 2002; Imabayashi et al. 2003).

Heparin binding properties

YKL-40 contains a single putative heparin binding site (location 144-147) (Malinda et al. 1999, Nishikawa et al. 2003) and binds heparin with an affinity greater than fibronectin (Millis et al. 1985; Millis et al. 1986; Shackelton et al. 1995). YKL-40 may interact with heparin-like molecules in the ECM or on the cell surfaces. Fusetti et al. (2003) have shown using crystallization methods that heparan sulfate and not heparin is a more likely physiological ligand of YKL-40. The physiological role of heparan sulfate proteoglycans are highly diversified, including cell adhesion, proliferation, migration, differentiation, growth factor and cytokine action, tissue morphogenesis/organogenesis, tissue remodeling and wound healing (Iozzo et al. 1994; SundarRaj et al. 1995). YKL-40 may function by interacting with components of the cell surface or the ECM and one physiologic ligand for YKL-40 could be perlecan (a heparan sulfate proteoglycan) which is a component of basement membranes and expressed in the ECM of many tissues including cartilage, liver, and cancer. Proteins anchored on glycosaminoglycan side chains of heparan sulfate proteoglycans serve a variety of functional purposes, from simple immobilization or protection against degradation to modulation of distinct biological activities (Lindahl et al. 1998; Perrimon et al. 2000). Transient and selective expression of heparan sulfate proteoglycans is elucidated as to deliver growth factors (e.g. FGF, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF)) to their appropriate receptors on fibroblasts or endothelial cells for signaling new tissue growth during the repair processes (Clasper et al. 1999).

Chitin binding properties

YKL-40 has high amino acid sequence homology to bacterial chitinases (Hakala et al. 1993; Johansen et al. 1993 I) and strong binding affinity for chitin (Hakala et al. 1993; Renkema et al. 1998; Houston et al. 2003), but YKL-40 lacks chitinase activity (Hakala et al. 1993; Renkema et al. 1998). Chitooligosaccharides bind to YKL-40 with μM affinity (Houston et al. 2003), and oligomeric chitin could be a physiological ligand for YKL-40, although binding of other carbohydrate polymers cannot be excluded. The chitinase activity is dependent on aspartic acid (D) and glutamic acid (E) at the end of the conserved catalytic center DxxDxDxE sequence motif (Watanabe et al. 1993; Watanabe et al. 1994; van Aalten et al. 2001; Bokma et al. 2002). The essential aspartic acid is conserved in YKL-40, *Drosophila* DS47 and IDGF1, and in all the "mammalian chitinase-like proteins" except in mouse Ym1, where it is replaced by asparagine. The essential glutamic acid is replaced by leucine in YKL-40 and OGP, by isoleucine in YKL-39, and by glutamine in mouse Ym1, *Drosophila* DS47 and IDGF1. None of these proteins have chitinase activity. Of the known "mammalian chitinase-like proteins" only chitotriosidase (a protein expressed by macrophages and neutrophils) (Hollack et al. 1994; Boot et al. 1995; Boussac et al. 2000) and AMCase (a protein expressed in the gastrointestinal tract and lung) (Boot et al. 2001) have chitinase activity and both have glutamic acid in the essential position. A single amino acid substitution in the catalytic domain of chitotriosidase, generating the same amino acid

sequence as in YKL-40, is followed by the loss of hydrolytic activity and retains the capacity to bind chitin (Renkema et al. 1998). In plants chitinases are believed to form part of innate immune system important for host defense against invading pathogenic bacteria and fungi and plants produce high amounts of chitinases under conditions of stress (Collinge et al. 1993; Sahai et al. 1993). It has been suggested that human chitotriosidase and AMCase play a role in host defense through degradation of chitin-containing cell walls of fungal pathogens (Boot et al. 2001). YKL-40 is secreted by macrophages and neutrophils, which serve in the primary defense mechanisms against invading pathogens. It has therefore been suggested that YKL-40 could act as a opsonin with a role in the immune response (Renkema et al. 1998), and that YKL-40 could act as a chitin sensor, switching on innate defenses, helping to direct macrophages to the site of invasion and to regulate the inflammatory response as a consequence of infection (Houston et al. 2003). Another of the "mammalian chitinase-like proteins", Ym1/ECF-L, has been proposed to have a role in directing components of the immune system to the site of nematode infections (Owashi et al. 2000).

Chitinases catalyze the hydrolysis of β-1,4-N-acetylglucoside linkages in chitin, a homopolysaccharide that consists of repeated N-acetyl-β-(1,4-linked) D-glucosamine (2-deoxy-2acetamino-D-glucose). Chitin is the principal structural component of the cell walls of plants, algae, fungi and bacteria, the microfilarial sheath of parasitic nematodes, of the shells or cuticles of arthropods, the lining of guts of many insects, in nematodes, mollusk, in worms and in the exoskeleton of fish and vertebrates (Flach et al. 1992; Araujo et al. 1993; Debone et al. 1994; Shahabuddin 1994). Although chitin is not found in mammals, YKL-40 may interact with an so far unknown endogenous compounds with chitin-like motifs that may exist in mammals. It has been found in vertebrates in an embryonic stage that short chito-oligosaccharides are used as primers for the synthesis of hyaluronan (Meyer et al. 1996; Semino et al. 1996; Varki 1996).

Effect on hyaluronan synthesis

Hyaluronan is a linear polysaccharide composed of repeating disaccharide units of N-acetyl glucosamine and D-glucoronate linked together by alternating β(1,4) and β(1,3) glycosidic bonds. Hyaluronan is located in the ECM of many tissues and is synthesized by articular chondrocytes, synovial cells in the inflamed synovial membrane, smooth muscle cells in injured vessels, hepatic stellate cells in liver fibrosis, fibroblasts in skin tissue and by cancer cells. Hyaluronan has multiple physiological roles and has been connected with embryogenesis, morphogenetic processes, cell proliferation and tissue remodeling and is involved in acute and chronic inflammatory processes (Laurent 1998; Lee et al. 2000). The expression of YKL-40 is related to similar events as hyaluronan (see Chapter 4 and 5) and the function of YKL-40 may be linked to the functions of hyaluronan. It is not known if YKL-40 binds hyaluronan, but YKL-40 has two potential hyaluronan binding motifs (location 147-155 and 369-377) (Malinda et al. 1999; Nishikawa et al. 2003). YKL-40 may recognize hyaluronan, or its precursor, as a substrate in the ECM and interfere with its synthesis, which could affect local hyaluronan levels and consequently influence the extent of cell adhesion and migration during the tissue remodeling processes that take place during inflammation, fibrosis, atherogenesis and metastasis.

REGULATION OF YKL-40

Effect of different extracellular matrix (ECM)

Several studies indicate that changes in ECM are related to changes in YKL-40 synthesis. Changes in the ECM environment of chondrocytes seem to affect YKL-40 production by these cells. Chondrocytes propagated under culture conditions undergo phenotypic changes both in morphology (i.e. the loss of the chondrocyte spherical shape and the acquisition of an elongated fibroblast-like morphology) and in gene expression pattern. The morphologic alterations of the chondrocytes are accompanied by profound biochemical changes, including loss of the cartilage-specific phenotype, as evidenced by an arrest of the synthesis of the cartilage-specific collagens (types II, IX, and XI) and proteoglycans (aggrecan), initiation of synthesis of the interstitial collagens (types I, III, and V), and increase in the synthesis of fibroblast-type proteoglycans at the expense of aggrecan. The chondrocyte phenotype can be re-expressed in the cells by culturing them in suspension, in agarose, with alginate beads, or on a hydrogel substrate (Benya et al. 1982; Bonventure et al. 1994; Haüselmann et al. 1992 and 1994; Freed et al. 1993; Reginato et al. 1994). These changes in the biosynthetic profile of dedifferentiated chondrocytes resemble some of the phenotypic changes displayed by OA chondrocytes, and the matrix they produce is similar to that synthesized by chondroprogenitor cells (Benya et al. 1978; Kosher et al. 1986; Aigner et al. 1993, 1997 and 1999). Microarray gene expression analysis of human fetal chondrocytes cultured either under conditions that allow them to preserve their differentiated phenotype or under conditions that lead to their dedifferentiation show that YKL-40 was overexpressed 4.4 fold in dedifferentiated human fetal chondrocytes compared to differentiated chondrocytes (Stokes et al. 2002). Normal cartilage explant cultures produce low levels of YKL-40 during the first days of culture but after a few days YKL-40 secretion increases. Stimulation of YKL-40 production is also generated by the trauma of cartilage resection and by removal of chondrocytes from their native ECM environment. Freshly isolated chondrocytes from normal cartilage do not secrete YKL-40 during the fist days of monolayer culture, but in first-passage monolayer cultures the cells produce >300-fold higher levels of YKL-40 compared to primary chondrocyte cultures. If chondrocytes are cultured as monolayer or in suspension with methacrylate, the cells first have to make cell-to-cell contact and then they produce an ECM. Chondrocytes cultured in these two systems secrete large amounts of YKL-40 and the production is prevented if the chondrocytes are cultured in alginate, where the cells already are surrounded by an ECM (Johansen et al. 2001c VII).

The production of YKL-40 is also increased many fold after monolayer cultures of smooth muscle cells form nodules. This YKL-40 synthesis is not the result of the absence of cell proliferation, but is closely linked to nodule formation (Millis et al. 1985). The expression of YKL-40 mRNA from mouse mammary tissue at different stages of functional differentiation shows that YKL-40 is expressed at very low levels prior to and during pregnancy and lactation, but its expression increases many fold during mammal gland involution (Morrison et al. 1994) which is characterized by increased tissue remodeling.

Effect of cytokines and growth factors

Regulatory studies of YKL-40 expression by cytokine and growth factors are sparse. IL-1β inhibited YKL-40 mRNA expression and secretion by human monolayer chondrocyte and cartilage explant cultures (Johansen et al. 2001c, VII) but has no effect on YKL-40 secretion by guinea pig chondrocyte cultures (De Ceuninck et al. 1998). Transforming growth factor β (TGFβ) reduces YKL-40 mRNA expression in human chondrocytes (Hakala et al. 1993; Johansen et al. 2001c, VII), as well as the synthesis of YKL-40 from human chondrocytes (Johansen et al. 2001c, VII) and guinea pig chondrocytes (De Ceuninck et al. 1998). YKL-40 secretion by freshly isolated chondrocytes (Johansen et al. 2001c, VII) is stimulated by IL-6 (a cytokine with important roles in the inflammatory process (Xing et al. 1998)), IL-17 (a proinflammatory cytokine with a role in joint inflammation and acts in synergy with IL-1β and tumor necrosis factor α (TNFα) (Miossec 2003)), and IL-18 (a cytokine contributing to cartilage degradation (Olee et al. 1999)). IGF-I and IGF-II stimulate YKL-40 synthesis by guinea pig chondrocytes (De Ceuninck et al. 1998) but not by human chondrocytes (Hakala et al. 1993; Johansen et al. 2001c, VII). The controversy with the effective-

ness of IL-1β and IGF-I on human and guinea pig chondrocytes may rest on differences in the species, donor age, doses or tissue conditions. TNFα, platelet-derived growth factor (PDGF), bFGF, $1,25(OH)_2D_2$, dexamethasone and serum have no effect on YKL-40 production by monolayer chondrocyte and cartilage explant cultures (De Ceuninck et al. 1998; Johansen et al. 2001c, VII). The regulation *in vitro* of YKL-40 secretion by human synovial cells was not influenced by IL-1β and TNFα (Nyirkos et al. 1990).

CONCLUSIONS AND FUTURE PERSPECTIVES

YKL-40 can be categorized as a member of the glycosyl hydrolase family 18 that includes at least eight "mammalian chitinase-like proteins". The conservation of exon-intron boundaries of the genes of the "mammalian chitinase-like proteins" and their chromosomal location, amino acid sequence homology and structural similarities suggest that the genes of these members of the "mammalian chitinase-like protein family" have evolved from chitinases to acquire new properties. YKL-40 is a secreted protein produced in humans by activated macrophages and neutrophils in different tissues with inflammation and increased remodeling of the ECM, by arthritic or injured chondrocytes, by fibroblast-like synovial cells, by vascular smooth muscle cells, and probably by hepatic stellate cells. The complete *in vivo* biological functions of YKL-40 remains to be established, but it may have a function in inflammation and remodeling of the ECM. *In vitro* studies have demonstrated that YKL-40 exerts growth factor properties on fibroblasts, chondrocytes, fibroblast-like synovial cells, and endothelial cells, and promotes vascular smooth muscle cell attachment, spreading and migration. It has been hypothesized that YKL-40 protects cells from undergoing apoptosis. YKL-40 may also have a role in hyaluronan synthesis and, due to its heparin/heparan sulfate binding properties, its function could be linked to changes in the ECM. The elucidation of the biological function of YKL-40 is an important objective of future studies and YKL-40 transgenic and knock-out mice will hopefully be developed. Also research on YKL-40 potential receptor(s), the regulation of YKL-40 expression, and the evolutionary relationship of the different members of the "mammalian chitinase-like protein family" could give insights into the physiological role of YKL-40 and its family members.

2. AIM

The purpose of this thesis was to determine if serum YKL-40 is a clinically useful biomarker of disease activity and prognosis in human disease. At the time these studies commensed, it had been established that YKL-40 is a major secreted protein of two cell types, articular cartilage chondrocytes and breast cells, when these cells are engaged in remodeling their ECM, but is not significantly expressed by either cell type under normal physiological circumstances. These observations showed that YKL-40 expression could be a biomarker for unique physiological states, and guided the selection of human diseases for study.

To explore if YKL-40 is a new biomarker, the YKL-40 protein expression in different tissues and the serum concentration of YKL-40 were determined in patients with selected acute and chronic diseases characterized by inflammation, remodeling of the ECM, development of fibrosis, and cancer. The following questions were addressed:

1. Is the occurrence of YKL-40 protein expression in human tissues characterized by inflammation, remodeling of the ECM, fibrosis and cancer?
2. Is the serum concentration of YKL-40 related to disease activity and prognosis in patients with diseases characterized by inflammation, remodeling of the ECM, fibrosis and cancer?
3. Can the serum concentration of YKL-40 provide new and more specific information of disease activity and prognosis compared to conventional parameters of disease activity in patients with diseases characterized by inflammation, remodeling of the ECM, fibrosis and cancer?
4. Can the serum concentration of YKL-40 give information on the pathophysiology and pathogenesis of diseases characterized by inflammation, remodeling of the ECM, fibrosis and cancer?

3. METHODS FOR DETERMINATION OF YKL-40 IN TISSUES AND BODY FLUIDS

MICROARRAY CDNA ANALYSIS

Several studies have evaluated YKL-40 gene expression using microarray gene analysis. Different human tissues and cells are tested: monocytes and macrophages (Hashimoto et al. 1999a; Suzuki et al. 2000), fetal chondrocytes (Stokes et al. 2002), gliomas (Lal et al. 1999; Markert et al. 2001; Tanwar et al. 2002), thyroid carcinomas (Huang et al. 2001), extraskeletal myxoid chondrosarcoma (Sjögren et al. 2003), hippocampus tissue (Chung et al. 2003), *Helicobacter*-infected murine stomachs (Mueller et al. 2003) and murine ovariectomiced chorioretinal tissue (Sharon et al. 2002; Rakic et al. 2003).

IN SITU HYBRIDIZATION

In situ hybridization studies have evaluated YKL-40 mRNA expression in frozen and formalin-fixed paraffin-embedded tissues in different human tissues: synovial membrane from RA patients (Kirkpatrick et al. 1997), normal and atherosclerotic coronary arteries and aorta (Boot et al. 1999), cartilage and osteophytic tissue from OA patients and healthy adults and from normal and fetal bone (Connor et al. 2000).

IMMUNOHISTOCHEMICAL ANALYSIS

Several immunohistochemical procedures for the detection of YKL-40 protein expression in biopsies of human tissues have been described using well known immunohistochemical methods for frozen or formalin-fixed paraffin-embedded tissues. An affinity purified rabbit antibody against human YKL-40 (Johansen et al. 1997, 1999a V, 2000a VI; Volck et al. 1998, 1999, 2001; Kawashaki et al. 2001) or a mouse monoclonal antibody against human YKL-40 (Baeten et al. 2000; Johansen et al. 2001c VII) were used as primary antibody. Different human tissues are evaluated: liver (Johansen et al. 1997, 2000a VI), bone marrow (Volck et al. 1998), inflamed arteries (Johansen et al. 1999a V), cartilage (Volck et al. 1999, 2001; Johansen et al. 2001c VII; Kawashaki et al. 2001), synovial membrane (Baeten et al. 2000; Volck et al. 2001), peripheral blood mononuclear cells (Baeten et al. 2000) and atheroslerotic vessels (Nishikawa et al. 2003).

RADIO- AND ENZYME-LINKED IMMUNOASSAYS
FOR THE DETERMINATION OF YKL-40

Three immunoassays for the measurement of human YKL-40 in body fluids (serum, plasma, synovial fluid, cerebrospinal fluid) and conditioned human cell culture media are described in the literature (Johansen et al. 1993 I; Harvey et al. 1998; Vos et al. 2000b). The first human YKL-40 assay was a radioimmunoassay (RIA) using a rabbit polyclonal antibody against human YKL-40 (Johansen et al. 1993 I). The assay runs over two days, involves 20 hours incubation at room temperature and requires sample dilution. The sensitivity of the RIA was 10 μg/l and the recovery 100.3%. The intraassay coefficient of variation (CV) was < 6.5%. The short term interassay CV (during a 5 months period) was <12% (Johansen et al. 1993 I) and the long term interassay CV (during a 5 years period) was <15% (personal observation). A two-site, sandwich-type enzyme-linked immunoassay (ELISA) for measurement of human YKL-40 was later developed and is commercially available from Quidel (CA, USA) (Harvey et al. 1998). This assay uses streptavidin-coated microplate wells, a biotinylated-Fab monoclonal mouse antibody against human YKL-40 (capture antibody) and an alkaline phosphatase-labeled polyclonal rabbit antibody against human YKL-40 (detection antibody). Bound enzyme activity is detected with p-nitrophenyl phosphate as

substrate. The ELISA is finished within 4 hours and involves three 1-hour incubation steps, is carried out at room temperature and does not require sample dilution (only if the concentration of YKL-40 in the sample is very high). The sensitivity of the ELISA was 8 μg/l and the recovery 102%. The intraassay CV was < 3.6%. The short term interassay CV (during a 11 days period) was < 3.7% (Harvey et al. 1998) and the long term interassay CV (during a 5 years period) is < 8.6% (personal observation).

The YKL-40 protein used for standards and antigen for antibody production in the RIA and ELISA and for tracer in the RIA was purified from serum-free conditioned medium of monolayer cultures of the human osteosarcoma cell line MG63 (Johansen et al. 1993 I; Harvey et al. 1998) by a modification of the heparin-affinity chromatography method described to purify YKL-40 (Rejman et al. 1988; Nyirkos et al. 1990). MG63 cells (obtained from the American Type Culture Collection, Rockville, MD) are easily cultured in serum free media and at confluence these cells secrete large amounts of YKL-40 (Johansen et al. 1992). Preparations of polyclonal or monoclonal antibodies were produced by routine procedures (Johansen et al. 1993 I; Harvey et al. 1998), but the specific epitopes of human YKL-40 recognized by these antibodies are unknown. Human YKL-40 concentrations in blood (serum or EDTA plasma), synovial fluid and cerebrospinal fluid (described in Chapter 3 and 4), and in conditioned medium of human cell cultures (Johansen et al. 2001c VII) can be determined using these methods. The YKL-40 ELISA is also useful for the measurement of serum YKL-40 levels in baboons (Mahanery et al. 1998) and cynomolgus macaques (Register et al. 2001). The YKL-40 RIA and ELISA can not detect mouse, rabbit, cow or swine YKL-40.

High correlation (Spearmans rho = 0.91, $p < 0.0001$) was found between the serum concentrations of YKL-40 determined by RIA and ELISA. The mean serum YKL-40^{ELISA}/YKL-40^{RIA} ratio was 0.479 calculated from 506 serum samples from 245 healthy adults, 112 RA patients, 37 OA patients and 112 patients with metastatic breast cancer (personal observation). The difference between serum YKL-40 levels using the two methods is probably explained by differences in the methods used to calculate YKL-40 protein concentration in the standards used in the two assays. The relative YKL-40 antigen recognition by the two assays was constant. In the following chapters and in the data presented in Figures 2-5 and Tables 1-4 the serum concentrations of YKL-40 determined by the YKL-40 RIA have been adjusted to YKL-40 ELISA results by multiplication with 0.479 for a better comparisons of the serum YKL-40 levels in the different studies.

Three other YKL-40 ELISAs have been developed but have only been used in a few clinical studies. Vos et al. (2000b) developed a human YKL-40 ELISA using plates coated with a mouse monoclonal antibody against human YKL-40 (capture antibody) and a horseradish peroxidase (HRP) labeled mouse monoclonal antibody against human YKL-40 (detection antibody). Rejman et al. (1989) developed an ELISA for YKL-40 determination in bovine mammary milk secretions during involution using plates coated with bovine YKL-40, a rabbit polyclonal antibody against bovine YKL-40 and HRP-technique. De Ceuninck et al. (2001b) developed an indirect competition ELISA for measurement of serum YKL-40 in guinea pigs using a polyclonal anti-guinea pig YKL-40 antibody produced in hens and extracted from the egg yolk. This assay can also determine rabbit YKL-40 but not rat or mice YKL-40.

SERUM/PLASMA CONCENTRATIONS OF YKL-40
IN HEALTHY SUBJECTS

The individual serum concentrations of YKL-40 in 245 healthy adults (aged 18-79 years) according to age is illustrated in **Figure 1**. The subjects are described by Johansen et al. (1996a III) using the YKL-40 RIA method and their serum YKL-40 level was later determined by YKL-40 ELISA in 1997. No difference in serum YKL-40 was found between gender, but there was a relation between serum YKL-40 and age (rho = 0.45, $p < 0.001$) with the highest levels in the elderly. All subjects were healthy without symptoms of disease and were not taking medicine at the time of blood sampling. They had normal serum levels of creatinine, albumin, lactate dehydrogenase (LDH), aspartate aminotransferase, alkaline phosphatase and bilirubin. These subjects were not followed prospectively and it is not known if some later developed cancer that was not clinically detectable at the time of blood sampling. Aging is associated with low-grade inflammation (Bruunsgaard et al. 2001) and the increase in serum YKL-40 in elderly healthy subjects may be due to low-grade inflammation. Serum YKL-40 in 476 healthy children (aged 7-17 years) was similar to the level in healthy young adults and there was no change in serum YKL-40 during puberty (Johansen et al. 1996a III).

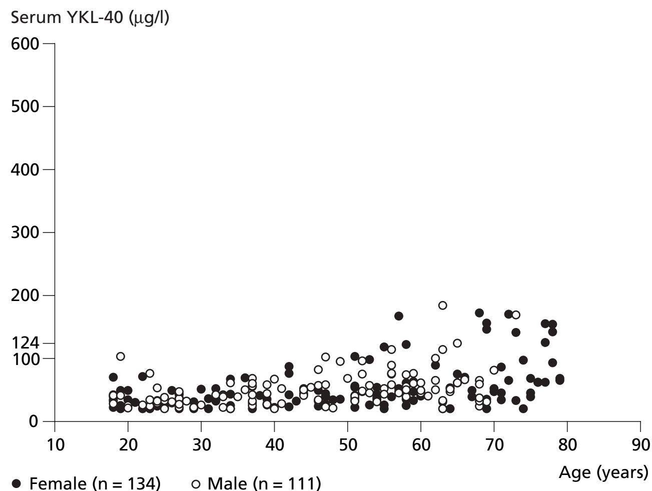

Figure 1. Individual serum YKL-40 concentrations in 245 healthy adults in relation to sex and age. The serum YKL-40 concentrations were determined by ELISA. The upper 95th percent limit of serum YKL-40 levels in these healthy adults is 124 μg/l.

There is good agreement between serum concentrations of YKL-40 in the two largest studies of healthy subjects (Johansen et al. 1996a III; Harvey et al. 1998) with a median serum YKL-40 of 43 μg/l (90% percentile = 95 μg/l; 95% percentile = 124 μg/l). The serum YKL-40 level in the study by Garnero et al. (2001) was higher, but these controls were older compared to the other studies of healthy subjects. In clinical studies of serum concentrations of YKL-40 in patients with different diseases it is important to compare serum YKL-40 in the patients with an age-corrected upper normal serum YKL-40 (e.g. 95th percentile).

Haemodynamic investigations with catherization of the femoral artery and renal vein indicated that the kidney is the main site of YKL-40 disposal (Johansen et al. 1997). The plasma concentration of YKL-40 in the renal vein was significantly lower than in the femoral artery both in subjects with normal liver function and in patients with chronic liver disease. Furthermore, YKL-40 can be detected in urine (personal observation). In healthy subjects there was no correlation between serum concentrations of YKL-40 and creatinine (Johansen et al. 1996a III). Whereas patients with severe renal diseases (i.e. requiring hemodialysis or peritoneal dialysis) had significantly elevated serum YKL-40 compared to healthy subjects (personal observation).

INDIVIDUAL VARIATION
IN SERUM YKL-40 CONCENTRATIONS

There was no circadian variability in serum concentrations of YKL-40 in samples collected 7 times during the day from 16 healthy subjects (aged 32-66 years) and 21 patients with RA (aged 30-75 years). The long time CV in serum YKL-40 was 5% in 30 healthy women (aged 24-62 years) who had serum samples collected 5 times with seven days intervals and subsequently again after 3 years (Johansen et al. manuscript in preparation).

STABILITY OF YKL-40 CONCENTRATIONS IN BLOOD AFTER VENIPUNCTURE

Several factors must be considered when handling blood samples for the measurement of YKL-40. The time interval between drawing of blood and centrifugation of blood stored at room temperature must be less than 3 hours for serum and 8 hours for EDTA plasma samples. Otherwise significant and not disease related elevations of YKL-40 are found in the serum and EDTA plasma samples left on the clot for a longer time when compared with YKL-40 concentrations in serum and EDTA plasma samples centrifuged within 1 hour after venipuncture. If the blood was stored at 4°C before centrifugation YKL-40 concentrations were stable in serum for 24 hours and in EDTA plasma for 72 hours (Høgdall et al. 2000b). Degranulation of neutrophils with release of YKL-40 from the specific granules is the most likely explanation for this time dependent increase in YKL-40 concentrations in serum and EDTA plasma. YKL-40 accumulated extracellularly in a time-dependent manner in standard erythrocyte components, and prestorage leukocyte depletion of whole blood prevented extracellular YKL-40 accumulation (Cintin et al. 2001). Repetitive freezing and thawing of serum samples up to 9 times had no effect on the serum YKL-40 (Johansen et al. 1993 I; Harvey et al. 1998; Høgdall et al. 2000b; De Ceuninck et al. 2001b; Vos et al. 2001b). YKL-40 concentrations in serum were stable in samples stored up to 5 days at room temperature (Johansen et al. 1993 I), up to 9 days at 4°C (Harvey et al. 1998), and at –20°C or –80°C for at least 8 years (personal observation). YKL-40 concentrations in corresponding serum and EDTA plasma samples were correlated (rho = 0.98, $p<0.001$), but YKL-40 was significantly higher in serum compared to EDTA plasma with a YKL-40 serum/EDTA plasma ratio of 1.4 (Johansen et al. 1993 I; Høgdall et al. 2000b). This is probably caused by a small release of YKL-40 from activated neutrophils during the coagulation process. In this thesis there will no discrimination between YKL-40 in serum and plasma samples, since the serum or plasma concentrations of YKL-40 in the patients were accordingly related to the serum or plasma concentrations of YKL-40 in healthy subjects.

CONCLUSIONS AND FUTURE PERSPECTIVES

The in-house YKL-40 RIA and the commercial YKL-40 ELISA are both satisfactory methods for measurement of serum concentrations of YKL-40 in terms of reliability, reproducibility and stability. YKL-40 is detectable in serum of apparently healthy subjects and increases with older age. Puberty, a physiological condition with increased remodeling of ECM, does not result in increased serum YKL-40 levels. Most of the circulating YKL-40 in healthy subjects probably originates from activated macrophages and neutrophils. The high serum YKL-40 in some elderly healthy subjects may be due to low-grade inflammation or an undiscovered disease that influences serum YKL-40 levels. Circulating YKL-40 seems to be cleared by the kidneys, but studies are needed to determine the metabolism of circulating YKL-40, its circulating half-life and if YKL-40 is bound to substances in blood.

An automated test for determination of YKL-40 in serum will hopefully be developed in order to decrease expenses of a serum YKL-40 measurement. It is also important to develop an ELISA for determination of YKL-40 in mouse or rat for functional and pharmaceutical studies of YKL-40 in these animals.

4. YKL-40 IN NON-MALIGNANT DISEASES CHARACTERIZED BY INFLAMMATION, REMODELING OF THE EXTRACELLULAR MATRIX OR DEVELOPMENT OF FIBROSIS

YKL-40 is expressed and secreted by macrophages, neutrophils, fibroblast-like synovial cells, chondrocytes, vascular smooth muscle cells and hepatic stellate cells. It has been hypothesized that YKL-40 has a role in acute and chronic inflammation and in pathological conditions leading to tissue fibrosis. In this Chapter it is explored if determination of serum YKL-40 has a clinical value as a biomarker of disease activity and prognosis in patients with selected acute and chronic diseases characterized by inflammation, remodeling of the ECM or development of fibrosis. What is a biomarker? In 2001 the "Biomarkers and Surrogate Endpoint Working Group" agreed on a classification system and definitions for biomarkers (Atkinson et al. 2001). A "Biomarker" (Biological marker) was defined as: "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention".

4.1. INFECTIOUS DISEASES

More than 75% of patients with *Streptococcus pneumoniae* pneumonia (Nordenbaek et al. 1999) and *Streptococcus pneumoniae* bacteremia (Kronborg et al. 2002) had elevated serum concentrations of YKL-40 compared with age-matched healthy subjects (**Table 1**). The peak in serum YKL-40 in patients with community-acquired *Streptococcus pneumoniae* pneumonia requiring hospitalization was seen on day 1 after hospitalization. Treatment with antibiotics of these patients resulted in decreases in serum YKL-40, reaching normal level in most patients within 7-14 days. The serum C-reactive protein (CRP) level reached normal range a few days later than serum YKL-40 (Nordenbaek et al. 1999). Patients with atypical pneumonia or *Haemophilus influenzae* had normal serum YKL-40 (Nordenbaek et al. 1999). YKL-40 could also be detected in the bronchioalveolar lavage fluid (BAL) from patients with tuberculosis (personal observation).

In patients with *Streptococcus pneumoniae* bacteremia the serum YKL-40 level was associated with the severity and fatal outcome of the disease (Kronborg et al. 2002). Serum YKL-40 was higher in patients who needed hemodialysis, pharmacological treatment of hypotension and mechanical ventilation compared to patients without the need of intensive supportive treatment. Multivariate Cox regression analysis (including serum YKL-40, cerebral symptoms, mechanical ventilation, pharmacological treatment of hypotension and hemodialysis) showed that high serum YKL-40 at time of diagnosis of *Streptococcus pneumoniae* bacteremia was an independent prognostic variable of poor prognosis in terms of survival from *Streptococcus pneumoniae* bacteremia. In the same patients serum CRP was not a prognostic marker of survival. If also the plasma concentration of soluble urokinase receptor was included in the multivariate Cox analysis serum YKL-40 was not an independent prognostic variable (Wittenhagen et al. 2004).

Østergaard et al. (2002) have shown that YKL-40 was produced locally within the compartment of an infection. Patients with purulent meningitis and encephalitis had higher YKL-40 concentrations in cerebrospinal fluid as compared with the YKL-40 levels in patients with lymphocytic meningitis and patients without meningitis. In the few patients who died of the infection the cerebrospinal concentration of YKL-40 was higher compared to the patients who survived. The overlap between cerebrospinal fluid concentrations of YKL-40 in patients with bacterial meningitis and lymphocytic meningitis was large and YKL-40 cannot be used for diagnostic purposes of patients with meningitis. Patients with non-infectious spinal diseases (i.e. cervical spondylotic myelopathy, lumbal canal stenosis and lumbar disc herniation) had higher YKL-40 concentrations in cerebrospinal fluid compared to controls or patients with scoliosis (Tsuji et al. 2002), but not as high as patients with infectious diseases.

In patients with *Streptococcus pneumoniae* pneumonia followed during treatment with antibiotics the changes in serum YKL-40 were parallel to that of serum lactoferrin and NGAL (proteins located in the specific granules of neutrophils like YKL-40), but not to serum MPO (a protein located in the azurophil granules), and only partial parallel to that of the total numbers of neutrophils in blood (Nordenbaek et al. 1999). In patients with *Streptococcus pneumoniae* bacteremia serum YKL-40 was inversely correlated with the total

Table 1. Serum YKL-40 levels (µg/l) in patients with inflammation, tissue remodelling or fibrosis and % of patients with elevated serum YKL-40 .

Diagnosis	N	Serum YKL-40	High YKL-40 (%)#	Reference
Streptococcus pneumoniae pneumonia§	22	428[c] (57-4311)	82	Nordenbaek et al. 1999
Pneumonia unknown aetiology§	58	215[c] (52-2347)	79	
Streptococcus pneumoniae bacteremia	89	342[c] (20-20400)	76	Kronborg et al. 2002
Giant cell arteritis§	19	123[b] (30-431)	53	Johansen et al. 1999a V
Polymyalgia rheumatica§	8	76 (35-199)	38	
Ulcerative colitis	94	103[a] ± 83	67	Koutroubakis et al. 2003
Crohns disease	85	112[a] ± 84	69	
Ulcerative colitis, inactive	61	33 (11-213)	11	Vind et al. 2003
Ulcerative colitis, mild/moderate	52	46 (10-222)	17	
Ulcerative colitis, severe	51	59[c] (21-736)	29	
Crohns disease, inactive	92	43 (13-1156)	24	Vind et al. 2003
Crohns disease, mild/moderate	34	57 (12-189)	26	
Crohns disease, severe	37	59[c] (19-1128)	38	
Pulmonary sarcoidosis§	27	201[c] (51-479)	63	Johansen et al. 2005b
Systemic sclerosis	40	76[c] (24-584)	35	Montagna et al. 2003
Systemic sclerosis§	88	77[c] (24-805)	27	Nordenbæk et al. 2005
Fatty liver§	16	93 (24-195)	25	Johansen et al. 2000a VI
Viral hepatitis§	17	83 (53-182)	35	
Non-cirrhotic fibrosis§	31	158[c] (55-463)	61	
Posthepatitic cirrhosis§	10	204[c] (69-992)	80	
Alcoholic cirrhosis§	51	255[c] (39-2323)	90	
Chronic hepatitis C	49	78[c] (18-1276)	53	Nøjgaard et al. 2003b
Alcoholics, no fibrosis	17	147 (550)¤	–	Tran et al. 2000
Alcoholics, mild fibrosis	55	158 (800)¤	–	
Alcoholics, moderate fibrosis	15	402 (1500)¤	–	
Alcoholics, severe fibrosis	59	511 (1600)¤	–	
Alcoholics, no fibrosis§	43	72 (10-388)	26	Nøjgaard et al. 2003a
Alcoholics, slight fibrosis§	88	156[c] (31-2658)	64	
Alcoholics, moderate fibrosis§	146	186[c] (38-2658)	75	
Alcoholics, severe fibrosis§	59	201[c] (38-1532)	76	

Values are median (range) except when otherwise noted.
a: $p<0.05$, b: $p<0.01$ and c: $p<0.001$, compared with controls (Mann-Whitney test).
#: The percentage (%) of patients with elevated serum YKL-40 compared to the age-adjusted serum YKL-40 level in healthy subjects. The normal reference region was calculated on the log trasformed serum or plasma YKL-40 levels obtained from healthy subjects (aged 18-79 years; N = 260 for RIA values and N = 245 for ELISA values) (Johansen et al. 1996a III). The upper 95th per cent confidence limit was chosen for the limit and adjusted for age (Royston 1991).
§: RIA analysis (Johansen et al. 1993 I) but data corrected to ELISA values (YKL-40 ELISA = YKL-40 RIA X 0.479). All the other studies used the ELISA method (Harvey et al. 1998).
¤: Mean (upper value).

number of neutrophils in blood (Kronborg et al. 2002) and directly correlated with the soluble form of urokinase-type plasminogen activator receptor (expressed on different cell types including neutrophils, macrophages and lymphocytes) (Wittenhagen et al. 2004). In patients with bacterial meningitis a correlation was found between cerebrospinal fluid concentrations of YKL-40, lactoferrin and the number of neutrophils in cerebrospinal fluid and blood, but no such relationship was found in patients with lymphocytic meningitis or non-meningitis. YKL-40 is probably not released from the circulating neutrophils but is first released after the cells have reached the site of inflammation. Cerebrospinal fluid concentrations of YKL-40 from patients with bacterial meningitis, lymphocytic meningitis and non-meningitis were also correlated with the cerebrospinal fluid levels of neopterin (a protein secreted by macrophages, microgliae), indicating that YKL-40 is also secreted by activated macrophages in the cerebrospinal fluid (Østergaard et al. 2002).

Injection of healthy subjects with *Esherichia coli* endotoxin resulted in a significant increase in plasma YKL-40 within 2 hours and highest value is found at the 24 hours time-point after injection (Johansen et al. 2005a). The exact peak value in plasma YKL-40 was located between 8 and 32 hours after endotoxin injection, but was not further specified in the study, since blood samples were available at start and at 2, 4, 8, 24, and 32 hours after endotoxin injection. YKL-40 had a faster reaction time compared to CRP where the increase was first significant after 8 hours. Endotoxaemia is known to induce a marked increase in circulating TNFα and IL-6 already after 30 and 60 minutes with peak values at 90 minutes and 2-3 hours after endotoxin, respectively (Bundgaard et al. 2003; Krabbe et al. 2001). It is likely that regulatory relationships exist between TNFα, IL-6 and YKL-40 but the mechanisms are at present unknown.

The exact cellular source of the high serum and cerebrospinal fluid concentrations of YKL-40 in patients with infectious diseases is unknown but it probably originates from activated macrophages and neutrophils. YKL-40 is expressed by CD14+,CD16+ macrophages in patients with RA (Baeten et al. 2000) and this subpopulation of macrophages dominant often in sepsis (Fingerle et al. 1993). Macrophages, one of the most versatile cell type in the body, participate in a vast array of biological processes and are key mediators of both inflammatory functions (e.g. fighting infections) to tissue remodeling functions (e.g. wound healing) (Nathan 1987; Sunderkotter 1994). The diversity of macrophages' functional repertoire suggests that their differentiation and activation may be subject to the profound influence of environmental changes. Adherence to ECM stimulates monocytes to undergo differentiation into inflammatory or reparative macrophages and induces monocytes and macrophages to express macrophage colony-stimulating factor that promotes long-time survival, proliferation and phagocytic activities

(Morrissette et al. 1999; Vaday et al. 2000). As described earlier in Chapter 1 it has been suggested that YKL-40 may be a differentiation marker of macrophages since elevated YKL-40 expression was found when monocytes differentiated to macrophages (Krause et al. 1996; Rehli et al. 1997; Renkema et al. 1998; Rehli et al. 2003), and SAGE demonstrated that YKL-40 expression was increased 288 fold after stimulation of monocytes with GM-CSF, 182 fold after stimulation with M-CSF and 31 fold in lipopolysaccharide stimulated monocytes (Hashimoto et al. 1999a; Suzuki et al. 2000). However, the role of YKL-40 in acute inflammation is unknown. Inflammatory responses to infections require the emigration of monocytes, neutrophils and T-lymphocytes from the vascular system, through endothelium, and into the ECM surrounding the injured tissue. The alveolar macrophage is poised to respond to the usual daily exposure to bacteria that enter the terminal airways and is capable of initiating an inflammatory reaction in the event that the microbial burden is too large or too virulent to be contained by the resident macrophage alone (Nelson et al. 1995). These activated alveolar macrophages can also recruit neutrophils into the alveolus from the reserves within the pulmonary microvasculature where approximately 40% of the body's neutrophils are marginated (MacNee et al. 1990; Nelson et al. 1995).

The transition of neutrophils from the vasculature into the site of inflammation elicits remarkable changes in neutrophil behavior as the cells adhere to and migrate across ECM towards inflammatory foci and the ultimate in situ elimination of foreign microorganisms through phagocytosis, generations of reactive oxygen metabolites and release of microbicidal substances. Most of the steps in this process are dependent on the mobilisation of cytoplasmatic granules and secretory vesicles (Goetzl et al. 1996; Ganz et al. 1997; Owen et al. 1999; Vaday et al. 2000; Faurschou et al. 2003). YKL-40 is a constituent of the specific granules in neutrophils (Volck et al. 1998), and proteins in these granules often participate in antimicrobial activities. However, Østergaard et al. (2002) could not demonstrate that YKL-40 had antimicrobial activity.

Conclusions and future perspectives

YKL-40 can be regarded as an acute phase protein, since its serum concentration increased by more than 25% following an inflammatory stimulus (as defined by Kushner 1982). Patients with *Streptococcus pneumoniae* pneumonia or bacteremia had 8-10 fold higher serum concentrations of YKL-40 compared to healthy subjects. Serum or spinal fluid concentration of YKL-40 can to some extent indicate the severity and prognosis of a bacterial infection, and serum YKL-40 might add to the information of serum CRP in patients with severe acute bacterial infections. Activated macrophages and neutrophils are probably the major source of circulating YKL-40 in infectious diseases. However, the function of YKL-40 in the inflammatory foci of a bacterial infection is unknown and will hopefully be clarified in the future.

4.2. JOINT DISEASES

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a class II-associated autoimmune disease of unknown etiology characterized by symmetrical synovial inflammation of the joints. Most patients experience a chronic fluctuating course of the disease leading to joint damage with destruction of cartilage and periarticular bone resorption. Joint deformity and disability often follow this progressive joint destruction. The mortality of RA patients with long-term disease is increased compared with that of the general population (Gabriel et al. 1999). The ultimate goal of treating RA is to induce a complete remission, but this occurs infrequently and managing RA patients are therefore to prevent joint damage and loss of function and to decrease pain.

YKL-40 in relation to etiology of RA

A combination of genetic and environmental factors contribute to the initiation of RA. It is estimated from a twin population study that genetic factors account for 15% and non-genetic factors for 85% of the etiology of RA (Svendsen et al. 2001). Certain HLA-DRB1 alleles confer both predisposition to RA and an increased risk of disease severity. The best identified genes as risk factors for RA are the linkage to the major histocompatibility-complex class II antigens HLA-DRB1*404 and HLA-DRB1*401. Patients with HLA-DRB1*404 subtype exhibit greater disease severity, whereas patients with the HLA-DRB1*401 subtype have a milder form (Nepom et al. 1996; Thomson et al. 1999; Weyand et al. 2000a).

Autoimmune diseases represent a failure of development of tolerance to self-proteins and antigen presentation to T cells is a key event in several of the autoimmune diseases including RA. Antigen-activated CD4+ T cells in the synovial membrane provide mediators that stimulate monocytes, macrophages, and fibroblast-like synoviocytes to produce e.g. IL-1β, IL-6 and TNFα, and stimulate B cells to produce immunoglobulins, rheumatoid factor (RF) and osteoprotegerin ligands that stimulate osteoclastogenesis (Arend et al. 1995; van de Loo et al. 1995; Tak et al. 2000; Choy et al. 2001; Firestein 2003). The self-antigens may be immunogenic if they are novel or presented in an altered form via a nontraditional presentation pathway (Patil et al. 2001). No specific common antigen has been identified in the synovial membrane of RA patients. It is possible that a variety of antigens are involved in the T cell response and that the antigens presented to T cells vary during the course of the disease. A number of possible endogenous antigens have been identified, including type II collagen, citrullinated protein, heavy-chain-binding protein and YKL-40 (Bläss et al. 1999). YKL-40 is a candidate autoantigen in RA for the following reasons: 1) YKL-40 derived peptides, which bind with high affinity to the RA-associated HLA-DR1 and DR4, were recognized by peripheral T cells from RA patients and these T cells showed a proliferative response to YKL-40 peptides (Verheijden et al. 1997; Cope et al. 1999; Vos et al. 2000a); 2) HLA-DM-dependent presentation pathway was involved in the presentation of autoantigenic YKL-40 epitopes (Patil et al. 2001); 3) HLA-DR/YKL-40$^{263\text{-}275}$ complexes were expressed by dendritic cells in RA synovium (Steenbakkers et al. 2003; Baeten et al. 2004) and associated with histologic features of follicular synovitis and was specific for RA (Baeten et al. 2004); 4) YKL-40 induced a chronic relapsing arthritis in BALB/c mice, which clinically and histologically resembles RA; 5) This arthritis could be delayed and suppressed by intranasal administration of YKL-40 prior to immunization (Verheijden et al. 1997; Joosten et al. 2000); and 6) Nasal tolerization with YKL-40 had a beneficial effect on both inflammation and tissue destruction in collagen-induced arthritis in mice (Joosten et al. 2000). Kavanaugh et al. (2003) has shown in a small randomized, double blind, placebo controlled phase I/II study that 7 intravenous infusions with a soluble complex of native HLA-DR4 (β*0401) complexed to a 1311 Da peptide (corresponding to amino acid residues 263-275 of YKL-40) led to T cell inactivation and immunologic tolerance in patients with persistent RA. 67% of the patients who received the highest dose had a clinical response after 5 infusions compared with 14% in the placebo treated group. These findings suggest that YKL-40 may play a fundamental role in the pathophysiology of RA and that immunological tolerance of the protein may control disease activity in RA patients.

YKL-39, another mammalian chitinase-like protein, also induced arthritis in mice, and antibody production against recombinant YKL-39 was found in the immunized mice (Sakata et al. 2002). The precise existence of anti-YKL-40 antibodies in serum samples from RA patients is unknown. Sekine et al. (2001) found that only 1% of RA patients had detectable anti-YKL-40 antibodies and 8% had anti-YKL-39 antibodies.

YKL-40 expression in synovial membrane and cartilage of RA patients

The joints are enclosed in a strong fibrous capsule. Each articular

bone end within the joint is lined by a thin layer of articular cartilage, and the inner surface of the joint capsule is lined with the synovial membrane which consists of a surface layer of cells (synovial lining), a superficial microvasculature net, and a connective tissue substratum (subsynovium). The synovial lining in a normal joint is typical of 1-3 cell layers of which most are macrophages and fibroblast-like synovial cells. The subsynovium varies in components in different regions of the joint, comprising loose connective tissue, adipose tissue, dense fibrous tissue and a plexus of fine lymphatic vessels that are important for synovial fluid regulation and are the route by which proteins and macromolecules are removed from the joints. The ECM of the subsynovium is mainly composed of type I and type III collagens, sulfated glycosaminoglycans, hyaluronan, and structural glycoproteins. Articular cartilage is a multiphasic material with a fluid phase (water and electrolytes) and the ECM (composed of collagens, proteoglycans, hyaluronan, and noncollagenous proteins). The two main ECM components of articular cartilage, type II collagen and aggrecan, are almost cartilage specific. Proteins in articular cartilage are synthesized by chondrocytes, the only cells located in articular cartilage.

One of the earliest histological changes of synovial inflammation in early RA patients is an increased number of blood vessels (Tak et al. 2000). Angiogenesis (formation of new microvessels from the preexisting vasculature) is essential in maintaining and nourishing synovial tissue and has a central feature in synovial inflammation and pannus formation, and without angiogenesis leukocyte ingress could not occur (Koch et al. 1994, 1998, 2000; Paleolog et al. 1998a; Walsh 1999). Although synovial neoplasia is not associated with RA, parallels are found between tumors and the arthritic synovial membrane, with its features of hyperplasia, hypertrophy, inadequate apoptosis of the synovial-lining cells, oedema, angiogenesis, inflammation and invasivenes. Many factors known to promote angiogensis and proliferation of invasive tumors (Ferrara 2002; Bergers et al. 2003) are also produced by fibroblast-like synovial cells (Bucala et al. 1991; Remmers et al. 1991; Qu et al. 1994; Zvaifler et al. 1994; Firestein 2003), and the concept of a "transformed" phenotype has been applied to RA fibroblast-like synovial cells (Konttinen et al. 2000). Activated fibroblast-like synovial cells demonstrate aggressive growth and invasive activity, have increased expression of cell adhesion molecules and oncogenes, secrete proteolytic enzymes and cytokines, and carry intrinsic genetic defects that prevent them from undergoing apoptosis (Richlin et al. 1994; Firestein 2003). In early RA T cells and B cells infiltrate the synovial membrane and are also found in the synovial fluid, along with large numbers of neutrophils. In established RA, the synovial membrane becomes transformed into an inflammatory tissue, the pannus, that consists of macrophages, T and B cells, mast cells, endothelial cells and hyperplastic fibroblast-like synovial cells. The synovium from non-cartilage-pannus junction sites is also hypertrophic and edematous with accumulation of T and B cells, plasma cells, macrophages, neutrophils, mast cells, natural killer cells, and dendritic cells in the subsynovium and with macrophages and fibroblast-like synoviocytes in the synovial lining (Tak et al. 2000, Firestein 2003). Areas with granulomatous necrosis, fibrin deposition and fibrosis can be observed. Few neutrophils are found in the inflamed synovial membrane except at the cartilage-pannus junction, but large numbers of neutrophils traffic through the synovial lining layer into the synovial fluid. Neutrophils comprise >90% of the cells in synovial fluid from active RA patients and have a turnover rate of $>10^9$ cells per day in an inflamed knee joint (Hollingsworth et al. 1967).

IL-1β, IL-6 and TNFα (produced by activated macrophages and fibroblast-like synovial cells) are the key cytokines that drive inflammation in RA and have a primary role in the joint damage of RA. TNFα is an autocrine stimulator as well as a potent paracrine inducer of other inflammatory cytokines, including IL-1β, IL-6, IL-8 and granulocyte-monocyte colony-stimulating factor. IL-1β and TNFα are potent stimulators of macrophages, fibroblast-like synovial cells, neutrophils, chondrocytes and osteoclasts to release metalloproteinases (MMPs), other proteinases and tissue plasminogen activator. Furthermore, IL-1β and TNFα inhibit the synthesis of tissue inhibitors of metalloproteinases (TIMPs), collagen and proteoglycan and inhibit chondrocyte proliferation and induce cell death. This leads to degradation of cartilage and subchondral bone. Cartilage destruction is followed by an increase in the synthesis of TGFβ, IGF-I, bFGF, and bone morphogenic proteins (BMPs) which stimulate production of ECM, TIMPs and increase chondrocyte survival and replication. Bone erosions in RA patients is regulated by the receptor activator of nuclear factor (NF)-κB ligand (RANKL). RANKL is expressed by T cells and synoviocytes in response to TNFα and granulocyte-macrophage colony-stimulating factor and contribute to osteoclast maturation and activation (Walsh et al. 2004). IL-1β and TNFα also stimulate expression of adhesion molecules on endothelial cells and increase recruitment of neutrophils into inflammatory sites of the joints. The best studied anti-inflammatory cytokines are IL-10 (produced by monocytes, macrophages, B and T cells) and IL-4 (produced by CD4+ type 2 helper T cells). Both decrease IL-1β and TNFα, IL-4 participates in the differentiation and growth of B cells and inhibits the activation of type 1 helper T cells, and IL-10 inhibits the proliferation of T cells (Weiss 1989; Chatman et al. 1993; Moore et al. 1993, 1999; Smith 1994; Phillinger et al. 1995; Nagase et al. 1999; Van Meurs et al. 1999, Kotake et al. 1999; van Bezooijen et al. 1999; Gravallese EM et al. 2000; Tak et al. 2000; Choy et al. 2001; Firestein 2003).

Nyirkos et al. reported in 1990 that synovial cells obtained from the synovial membrane of RA patients at time of joint replacement secreted YKL-40 *in vitro*. In 1993 Hakala et al. demonstrated YKL-40 mRNA expression in inflammed synovial tissue and cartilage from RA patients but no expression in non-inflamed synovial membrane and normal cartilage. Recently two-dimensional polyacrylamide gel electrophoresis and mass spectrometry found that YKL-40 was a major protein of fibroblast-like synovial cells from RA patients with inflammed synovial membrane (Dasuri et al. 2004). Immunohistochemical analysis of RA synovial membranes have shown YKL-40 protein expression in mononuclear cells located in the stroma and along the synovial lining, and in the pannus YKL-40 antigen was also detected in the ECM (Baeten et al. 2000; Volck et al. 2001). Many of the cells with YKL-40 protein expression were CD68+ (i.e. macrophages) (Volck et al. 2001) and flow cytometric analysis showed that the YKL-40+ cells were also CD16+ with a variable expression of CD14, CD33 and HLA-DR+ (Baeten et al. 2000). Not all mononuclear cells in the synovial membrane of RA patients were YKL-40+. In some areas of the synovial membrane most of the mononuclear cells were YKL-40+ and in other areas no cells were YKL-40+. The number of YKL-40+ mononuclear cells was related with the degree of synovial inflammation, a severely inflamed synovial membrane had more YKL-40+ cells compared to a less inflamed membrane (Volck et al. 2001). The number of YKL-40+ cells in the synovial lining layer of the inflammed synovial membrane from RA patients correlated with a radiological score reflecting joint destruction (Baeten et al. 2000).

Immunohistochemical analysis of cartilage biopsies from knee joints of RA patients at time of total knee joint replacement showed YKL-40 protein expression in chondrocytes located in all layers of the cartilage, particularly by chondrocytes in the superficial and middle layer, here 25% of the chondrocytes had YKL-40 expression (Volck et al. 2001). No YKL-40 is found in the pericellular matrix or ECM of cartilage. The reason may be that YKL-40 is present in the cartilage ECM in a to low concentration to be detected by the antibodies or YKL-40 may be bound to macromolecules in a way that prevents its detection.

The functions of YKL-40 expression by macrophages, neutrophils, fibroblast-like synoviocytes, and chondrocytes in the arthritic joint are unknown. YKL-40 stimulates the proliferation rate of synovial cells and chondrocytes *in vitro* (De Ceuninck et al. 2001a;

Recklies et al. 2002) indicating an autocrine function, and YKL-40 is suggested to be a differentiation marker in chondrocytes (Imabyayashi et al. 2003) and may protect the cells from undergoing apoptosis (see Chapter 1). It is also possible that YKL-40 in the hypoxic arthritic joint stimulates angiogenesis. YKL-40 is a growth factor of vascular endothelial cells (Malinda et al. 1999; Nishikawa et al. 2003) and is up-regulated in cancer cells by hypoxia (Junker et al. 2005b). Hypoxia is a potent stimulus for angiogenesis and tumor growth (Harris 2002), and the RA synovial microenvironment is also often ischemic and hypoxic (Mapp et al. 1995; Bodamyali et al. 1998).

Biomarkers of disease activity and prognosis in RA patients

A major problem in facing decisions for treatment of RA patients is that no reliable predictive parameters exist for the disease course of the individual patient. The American College of Rheumatology (ACR) 1987 classification criteria for RA (Arnett et al. 1988) do not help in identifying RA patients with a high risk of severe disease. HLA-DRB1*04 alleles are predictors of development of bone erosions in patients with established RA (Wagner et al. 1997), and this has also been found in some (Combe et al. 2001; Goronzy et al. 2004) but not in all studies of patients with early RA (Wagner et al. 1997; Möttönen et al. 1998; Harrison et al. 1999). It is not yet recommended to use genetic markers as either diagnostic or prognostic criteria of RA patients. Poor prognosis of RA patients is suggested by bone erosions at time of diagnosis, high IgM RF, increased erythrocyte sedimentation rate (ESR) and serum CRP (Brennan et al. 1996; Young et al. 1997; Harrison et al. 2000; Scott 2000; Bukhari et al. 2001; Combe et al. 2001; Landewe et al. 2002; Visser et al. 2002, Goronzy et al. 2004), and variant mannose-binding lectin genotypes (Graudal et al. 2000a, 2000b; Ip et al. 2000). However, these parameters only explain some of the variation in progression of joint damage in RA patients.

ESR and CRP are the classical acute phase proteins used many years as biomarkers of inflammation to estimate disease activity in RA patients (Kushner 1991; Emery et al. 1993). ESR is an indirect reflection of the concentration in blood of many acute phase proteins (in particular fibrinogen), since its rate depends on the aggregation and subsequent fall of erythrocytes. Many abnormalities (e.g. microcytosis and anemia) influence the ESR and cannot be corrected for adequately. CRP, a member of the pentraxin protein family, is a pattern recognition receptor and probably a key component of the innate immune system, although its exact *in vivo* function is unknown (Kushner 1991; Gabay et al. 1999; DuClos 2000). CRP is not produced by cells in the arthritic joint but is secreted in the liver by hepatocytes in response to high circulating levels of IL-6, IL-1β and TNFα (Castell et al. 1990; Gauldie et al. 1992; Baumann et al. 1994). Serum CRP in RA patients is therefore a non-specific indirect measurement of synovial inflammation. The ACR RA guidelines recommend measurement of serum CRP at time of diagnosis of RA and periodically during treatment in order to evaluate disease activity and progression of joint destruction (ACR subcommittee on RA guidelines 2002). A high serum CRP over time in RA patients is associated with greater progression of joint destruction (Emery et al. 1993; Hassell et al. 1993; Van Leeuwen et al. 1994, 1997; Otterness 1994; Plant et al. 2000). However, high serum CRP at time of diagnosis is not a predictor of poor prognosis (Brennan et al. 1996; van Leeuwen et al. 1997; Green et al. 1999; Visser et al. 2002), and radiological progression of joint destruction can occur despite normal serum CRP. This is often the case in early RA patients.

Efforts have therefore been undertaken for the last two decades to find reliable biomarkers of disease prognosis in early RA patients and biomarkers of disease activity to be used during different treatment regimens and follow-up of RA patients. Potential biomarkers of joint inflammation and joint destruction in RA patients are proteins secreted by cells in the arthritic joint or molecules or fragments of connective tissue matrices of each of the 3 different joint tissues (synovial membrane, cartilage and bone) released into synovial fluid and serum during the process of synovial inflammation, and cartilage and bone degradation/remodeling. Vascular endothelial growth factor (VEGF) is a specific growth factor for endothelial cells and is secreted by activated macrophages and fibroblast-like synovial cells in the synovial membrane (Achen et al. 1998; Neufeld et al. 1999; Koch 2000, Ferrara et al. 2003). High serum levels of VEGF are found in patients with early and chronic RA and have been associated with severe disease activity and progression in joint destruction (Harada et al. 1998; Paleolog et al. 1998a, 2002; Ballara et al. 2001; Koch 2000). RA patients with high serum MMP-3 have a poorer prognosis and increased risk of joint destruction compared with RA patients with normal serum MMP-3 (Posthumus et al. 1999; Ribbens et al. 2000; Yamanaka et al. 2000). Serum TIMP-1 is found to be elevated (Yoshihara et al. 1995) or normal (Ishiguro et al. 1996; Keyszer et al. 1999) in RA patients but its prognostic value is unknown. Elevated IL-6 concentration in serum and synovial fluid are found in RA patients compared with controls, and is correlated with disease activity and joint destruction (Houssiau et al. 1988; Swaak et al. 1988; Dasgupta et al. 1992; Manicourt et al. 1993; Kotake et al. 1996; Desgeorges et al. 1997; Nishimoto et al. 2000). Serum IL-6 decreases after DMARD therapy (Dasgupta et al. 1992). Serum levels of hyaluronan (Goldberg et al. 1991; Hedin et al. 1991; Paimela et al. 1991; Laurant 2001) and the aminoterminal propeptide of type III procollagen (PIIINP) (Hørslev-Petersen et al. 1986, 1990) are also elevated in RA patients with active disease but are not sensitive markers of joint destruction. Potential biomarkers of cartilage remodeling are serum cartilage oligomeric matrix protein (COMP) and urinary concentrations of crosslinking telopeptides of type II collagen. Both parameters are reported to be related to variations in disease activity and joint destruction of RA patients (Forslind et al. 1992; Saxne et al. 1992; Månsson et al. 1995; den Broeder et al. 2002; Garnero et al. 2002b, 2002c). Potential biomarkers of bone remodeling are serum and urine levels of pyridinolins and the crosslinking telopeptides of type I collagen, serum RANKL, osteopontin and osteocalcin (Garnero et al. 2000, 2002b, 2002c). Many immunoassays, some of which are commercially available, are developed to measure these potential biomarkers in synovial fluid, serum/plasma, or urine. However, none are specific for RA or joint tissue, except the parameters reflecting type II collagen synthesis and degradation. None of these biomarkers are approved for routine use in daily clinical rheumatology practice as markers of disease activity and prognosis in RA patients. At present these biomarkers are only used for research purposes.

Ideally, a good biomarker to be used in RA patients for diagnosis, prognosis of joint destruction, prediction of effect of treatment and monitoring of disease activity would be a marker that is specific for RA, abnormal in RA patients with active disease (i.e. ongoing inflammation with destruction of cartilage and bone) and normal in patients with inactive disease. Such a marker is not available today and may not even exist. It is not likely that a single biomarker will be useful for exact determination of the complicated disease process in the arthritic joint of a RA patient. It is more likely that a combination of different biomarkers with imaging techniques (e.g. conventional radiography, magnetic resonance imaging (MRI), power Doppler ultrasonography (UL)) and clinical parameters of disease activity (e.g. the number of swollen and tender joints, disability index of the health assessment questionnaire (HAQ) score and visual analogue scale (VAS) of pain) will prove to be of clinical value for a more precise assessment of disease activity and prognosis in RA patients.

Synovial fluid concentrations of YKL-40 in RA patients

YKL-40 was detectable in synovial fluid from RA patients with concentrations from a few hundred nanograms to more than 5 micrograms per ml (Johansen et al. 1993 I; Kawasaki et al. 2001; Volck et al. 2001). The YKL-40 concentration in synovial fluid from RA pa-

Table 2. Synovial fluid (SF) and serum levels (μg/l) of YKL-40 in patients with rheumatoid arthritis and osteoarthritis.

Diagnosis	N	Median	(range)	Reference
SF YKL-40 (RA)§	29	1059	(338-2263)	Johansen et al. 1993 I
SF YKL-40 (knee OA)§	7	824	(352-1253)	Johansen et al. 1993 I
SF YKL-40 (late severe knee OA)§	15	931	(134-2498)	Johansen et al. 1996a III
SF YKL-40 (early knee OA)§	10	626	(240-3495)	Johansen et al. 1996a III
SF YKL-40 (severe hip OA)	19	886	(154-4728)	Kawasaki et al. 2001
SF YKL-40 (severe hip osteonecrosis)	21	2224	(810-5391)	Kawasaki et al. 2001
SF YKL-40 (failed hip arthroplasty)	5	3265	(2436-4009)	Kawasaki et al. 2001
SF YKL-40 (severe hip RA)	14	2120	(485-6850)	Volck et al. 2001
SF YKL-40 (severe knee OA)§	29	1190	(274-2600)	Volck et al. 2001
Serum YKL-40 (RA)§	29	70[b]	(21-350)	Johansen et al. 1993 I
Serum YKL-40 (knee OA)§	7	54	(32-114)	Johansen et al. 1993 I
Serum YKL-40 (late severe knee OA)§	37	90[b]	(32-789)	Johansen et al. 1996a III
Serum YKL-40 (early knee OA)§	17	54	(14-151)	Johansen et al. 1996a III
Serum YKL-40 (active RA)§	105	112[b]	(21-521)	Johansen et al. 1999b IV
Serum YKL-40 (inactive RA)§	20	81	(65-252)	Johansen et al. 1999b IV
Serum YKL-40 (early RA)§	51	62[b]	(17-431)	Johansen et al. 2000b
Serum YKL-40 (early RA)	76	98[c]	(21-408)	Johansen et al. 2001a
Serum YKL-40 (active RA)	56	126[c]	(20-525)	Harvey et al. 1998
Serum YKL-40 (inactive RA)	9	42	(20-125)	Harvey et al. 1998
Serum YKL-40 (OA)	27	104[c]	(20-720)	Harvey et al. 1998
Serum YKL-40 (early RA)	57	74	(50-102)#	Harvey et al. 2000
Serum YKL-40 (destructive RA)	166	99[c]		Garnero et al. 1999
Serum YKL-40 (non-destructive RA)	138	43		Garnero et al. 1999
Serum YKL-40 (knee OA)	67	121	(155)¤	Garnero et al. 2001
Serum YKL-40 (early RA)	191	110	(80)¤	Combe et al. 2001
Serum YKL-40 (early RA)	52	75[b]	(20-1085)	Peltomaa et al. 2001
Serum YKL-40 (RA)	72	150[c]	(7-525)	Matsumoto et al. 2001
Serum YKL-40 (hip OA)	45	68[a]	(33-343)	Conrozier et al. 2000
Serum YKL-40 (knee OA)	33	94[b]	(46)¤	Maciel et al. 2000
Serum YKL-40 (RA)[in house method]	47	31[a]	(10-375)	Vos et al. 2000
Serum YKL-40 (OA)[in house method]	51	21	(10-155)	Vos et al. 2000
Serum YKL-40 (severe RA of the knee)	12	87[b]	(20-218)	Volck et al. 2001
Serum YKL-40 (late severe knee OA)	31	73[b]	(26-565)	Volck et al. 2001
Serum YKL-40 (early OA)	29	112	(71)¤	Abe et al. 2004
Serum YKL-40 (late OA)	28	214	(193)¤	Abe et al. 2004
Serum YKL-40 (knee OA)	89	59	(35)¤	Pavelka et al. 2004

SF = synovial fluid. Values are median (range) except when otherwise indicated.
a: $p<0.05$, b: $p<0.01$ and c: $p<0.001$, compared with controls (Mann-Whitney test).
#) Median (interquartile range).
§) RIA analysis (Johansen et al. 1993 I) but data corrected to ELISA values (YKL-40 ELISA = YKL-40 RIA X 0.479).
All the other studies used the ELISA method (Harvey et al. 1998). ELISA[in-house] (Vos et al. 2000).
¤) Mean (SD).

tients with active disease was higher than the level in OA patients and patients with traumatic knee joint disease (Johansen et al. 1993 I, 1996 IIIa; Volck et al. 2001) (**Table 2**). The synovial fluid concentration of YKL-40 is determined by the secretion of YKL-40 from cells in the joint to the synovial fluid as well as by the clearance of YKL-40, which is unknown, from the joint cavity. YKL-40 in synovial fluid probably originates mainly from activated macrophages in the inflamed synovium and neutrophils in the synovial fluid. A small amount of YKL-40 may also originate from chondrocytes. The concentration of YKL-40 in synovial fluid from knee joints of RA and OA patients correlated with the volume of synovial membrane and joint effusion determined by MRI (rho = 0.64 and 0.59, $p<0.001$) (Volck et al. 2001). Furthermore, a relation was found between the number of YKL-40+ cells in the synovial membrane and the concentration of YKL-40 in synovial fluid (Volck et al. 2001). The relationship between the synovial fluid concentration of YKL-40 and a clinical index of synovial inflammation was low (Johansen et al. 1993 I). The concentration of YKL-40 in synovial fluid and serum was correlated (rho = 0.46-0.55), and 10-20 fold higher YKL-40 levels were found in synovial fluid compared to serum (Johansen et al. 1993 I; Volck et al. 2001). Synovial fluid concentrations of YKL-40 in RA patients were also correlated with ESR and serum CRP (rho = 0.62-0.68) (Volck et al. 2001), and with synovial fluid concentrations of IL-6 (rho = 0.47) and the elastolytic activity by live human monocytes/macrophages (rho = 0.58) (Johansen et al. 1993 I). No correlations were found between synovial fluid levels of YKL-40 and PIIINP, COMP, GAG and markers of specific granules in neutrophils (Volck et al. 2001). It is unknown if YKL-40 levels in synovial fluid are correlated with MMPs, TIMPs, VEGF and hyaluronan levels in synovial fluid.

Serum YKL-40 concentrations in RA patients in relation to disease activity

Twelve studies are published regarding serum YKL-40 in RA patients (Johansen et al. 1993 I, 1999b IV, 2000b, 2001b; Harvey et al. 1998, 2000; Garnero et al. 1999; Vos et al. 2000b; Combe et al. 2001; Matsumoto et al. 2001; Peltomaa et al. 2001; Volck et al. 2001) (Table 2). All reported elevated (1.4-3.5 fold) serum YKL-40 in patients with active RA compared to healthy controls, but a considerable overlap in serum YKL-40 existed between RA patients and controls. Patients with unclassified polyarthritis had normal serum YKL-40 (Johansen et al. 2000b). Serum YKL-40 was not related with disease duration (Johansen et al. 1999b IV; Vos et al. 2000; Matsumoto et al. 2001; Peltomaa et al. 2001) but increased as the functional disability of the patients became more severe (Matsumoto et al. 2001). **Figure 2** illustrates the individual serum YKL-40 levels in 395 RA patients according to disease activity and age (Johansen et al. 1999b IV; 2000b; Volck et al. 1999a). Patients with active RA had significantly higher serum YKL-40 than inactive RA patients and age-matched controls. No difference in serum YKL-40 was found

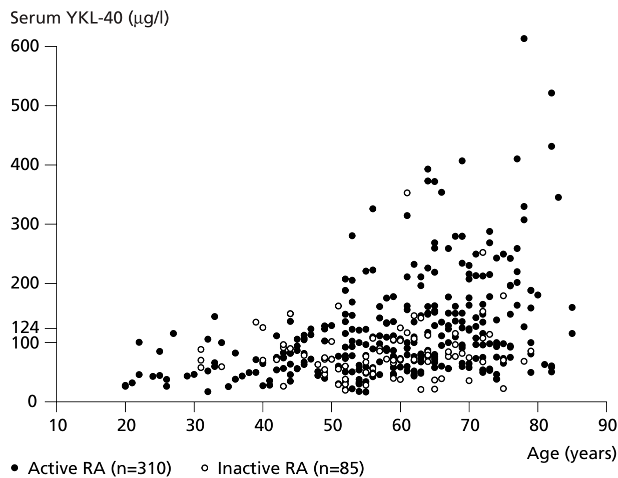

Figure 2. Individual serum YKL-40 levels in 395 RA patients (female/male: 305/90; median age = 60 years, range 20-85; median disease duration = 3.5 years, range 0.2-44). The patients had RA according to the ACR criteria (Arnett et al. 1988) and are described in 3 different studies (Johansen 1999b IV, 2000b; Volck et al. 1999a) and one unpublished. 310 of the RA patients had moderate to severe disease activity (defined by the presence of >2 groups of swollen joints and at least two of the following criteria: morning stiffness lasting >60 min, ESR >35 mm/hour and serum CRP ≥150 nmol/l) and 85 patients had inactive or mild disease activity (defined by the presence of ≤2 swollen joints, morning stiffness lasting <30 min, ESR ≤30 mm/hours and serum CRP <150 nmol/l). The serum YKL-40 levels were determined by RIA (Johansen et al. 1993 I) but the data was corrected to ELISA values (YKL-40 ELISA=YKL-40 RIA X 0.479). Patients with active RA had higher ($p<0.001$) serum YKL-40 (median 94 μg/l, range 20-613) than patients with inactive RA (median 72 μg/l, range 20-353). The upper 95th percent limit of serum YKL-40 in 245 healthy adults is 124 μg/l.

between inactive RA patients and controls. Serum YKL-40 was elevated (i.e. higher than the age-corrected upper 95th percentile of healthy subjects) in 37% of the RA patients with active disease and in 13% of the patients with inactive disease. 49% of patients with early RA (defined as a disease duration < 2 years) and active disease had elevated serum YKL-40 (Johansen et al. 2001b).

Low correlations were found between serum YKL-40 and an area-weighted swollen joint index (Johansen et al. 1999b IV) and the Ritchie Articular Index (RAI) (Peltomaa et al. 2001), and no correlation was found between serum YKL-40 and the degree of synovial inflammation expressed either as the total number of swollen joints, a knee index, or an articular index (Johansen et al. 1993 I, 1999b IV, 2001b). The reason may be that: 1) it is difficult precisely to measure the degree of synovial inflammation by counting the number of swollen joints; 2) a single large inflammed joint (e.g. the knee) contributes more to the amount of YKL-40 in serum than many small finger joints; 3) the metabolism and clearance of YKL-40 from the arthritic joint and blood is unknown; and 4) YKL-40 in serum may also originate from YKL-40+/CD14+/CD16+ PBMCs and from activated macrophages and neutrofils in other tissues.

Low correlations were found between serum YKL-40 and ESR and serum CRP levels in RA patients (rho = 0.33-0.60) (Johansen et al. 1993 I, 1999b IV, 2000b, 2001b; Harvey et al. 2000; Vos et al. 2000; Matsumoto et al. 2001; Peltomaa et al. 2001; Volck et al. 2001a; den Broeder et al. 2002), and high serum YKL-40 in RA patients were not always followed by elevations in ESR and serum CRP and vice versa. Approximately 70% of the patients with elevated serum YKL-40 had also high ESR or serum CRP (Johansen et al. 1999b IV) indicating that ESR, serum CRP and YKL-40 levels reflect inflammation of RA patients differently. The elevated serum YKL-40 level observed in some RA patients with active disease most likely originates from macrophages, neutrophils and fibroblast-like synovial cells in the arthritic joint, whereas ESR and CRP are not produced locally in the arthritic joint. Serum YKL-40 also correlated with serum PIIINP (Johansen et al. 1993 I, 1999b IV, 2001b), serum hyaluronan (2001b), blood Mø elastolysis (Johansen et al. 1993 I), serum IgM RF (Vos et al. 2000), serum pro-MMP3 (Johansen et al. 2001b), serum MMP1 and MMP3 (den Broeder et al. 2002), serum IL-6 (Johansen et al. 1993 I; Matsumoto et al. 2001), plasma VEGF (Klarlund et al. 2000), and inversely with serum IGF-I (Matsumoto et al. 2001). None of the correlations were high, only on the order of 0.25-0.55 (Spearmans rho). YKL-40 is a growth factor for endothelial cells (Malinda et al. 1999) and the relation between plasma VEGF and YKL-40 levels in RA patients may reflect a relation between serum YKL-40 and angiogenesis of the inflammed synovium. YKL-40 may also have an effect on PIIINP and hyaluronan synthesis by fibroblast-like synovial cells, since YKL-40 increases growth rates of fibroblastic cell lines (derived from human osteoarthritic synovium) and the number of rabbit synovial cells (De Ceuninck et al. 2001b; Recklies et al. 2002). No relations were found between serum YKL-40 and age, morning stiffness, grip strength, HAQ-, disease activity score (DAS), and doctor's and patient's VAS score (Johansen et al. 1999b IV; Vos et al. 2000; Peltomaa et al. 2001).

Flow cytometry has shown that the percentage of YKL-40+ PBMCs was higher in RA patients compared to patients with spondylarthropathy and healthy controls, but not different from patients with OA or liver cirrhosis. The number of YKL-40+ PBMCs correlated with ESR, serum CRP and YKL-40 levels. The YKL-40+ monocytes in peripheral blood from RA patients were different from "classic" monocytes and circulating dendritic cell precursors. The YKL-40+ cells were CD16+, had a dim expression of CD14 (Baeten et al. 2000) and resembled the CD14+,CD16+ monocyte population described by Ziegler-Heitbrock et al. (1996). Functional studies of CD14+,CD16+ cells indicate that these cells, compared with CD14++, CD16- monocytes, have acquired while in the circulation features in common with mature inflammatory tissue macrophages (see Chapter 1). The physiologic role of the YKL-40+, CD14+,CD16+ monocyte type is unknown. The frequency of CD14+,CD16+ monocytes was increased in RA patients compared to healthy subjects, and patients with increased frequency of CD16+ monocytes had active disease. It is unknown which factors drive the RA monocytes into this maturation pathway. It has been suggested that YKL-40+,CD16+ monocytes amplify the local autoimmune T cell response (Kawanaka et al. 2002), and that increased presence of YKL-40+ monocytes in RA patients does not reflect an abnormal cell population, but rather an activation of a specific differentiation pathway from normal monocytes to YKL-40+,CD16+ monocytes (Baeten et al. 2000). The phenotypic similarity between YKL-40+ mononuclear cells in blood and synovial tissue, the focal distribution pattern in the synovial lining, and the observation of solitary YKL-40+ mononuclear cells in synovial blood vessels and stroma suggest a migration of these cells between the peripheral blood and the synovial lining layer of RA patients (Baeten et al. 2000).

Serum YKL-40 concentrations in RA patients during different treatment regimens

Several studies have evaluated if serum YKL-40 in RA patients are influenced by treatment with different disease modifying anti-rheumatic drugs (DMARD's) or glucocorticoids (Johansen et al. 1999b IV, 2000b, 2001a, 2001b; Harvey et al. 1998; Charles et al. 1999; Volck et al. 1999a, 2001; Peltomaa et al. 2001). The mean decreases in serum YKL-40 (in % of initial value) were modest. Treatment with low doses of methotrexate (MTX) (7.5 to 10 mg p.o. once weekly) resulted in decreases in serum YKL-40 of 15% and 20% after 2 and 6 months of treatment, but after 12 months of MTX treatment no difference in serum YKL-40 was found compared to baseline levels (Johansen et al. 1999b IV). Early RA patients treated with sulphasalazine had decreases in serum YKL-40 of 17% after 3 months, 28% after 6 months and 29% after 24 months, whereas penicillamine had no effect on serum YKL-40 (Johansen et al. 2001b; Peltomaa et al. 2001). 12 months of leflunomide treatment reduced serum YKL-40 by 27% (Volck et al. 1999a). The mean % decreases in ESR and serum CRP in the same patients during

DMARD therapy were more pronounced than the decreases in serum YKL-40. Correlations existed between the % changes in serum YKL-40 and the % changes in ESR, serum CRP and PIIINP after one year of DMARD therapy of RA patients (ESR: rho = 0.50; CRP: 0.52; PIIINP: 0.38), but the correlations were not as high as the correlation between the % changes in ESR and serum CRP (rho = 0.75). The % changes in the number of swollen joints and serum YKL-40 in RA patients after one year of DMARD therapy were also correlated (rho = 0.46) and similar to the correlations between the % changes in the number of swollen joints, ESR and serum CRP (Johansen et al. 2001a).

ACR has developed criteria for defining improvement (Felson et al. 1995) and clinical remission (Pinals et al. 1981). It is a composite measure that can be used to categorize a patient as "improved" or "not improved" when specified disease manifestations at one point in time are compared with those at an earlier (e.g. baseline) time point. These criteria have been accepted for outcome assessment in clinical trials of RA patients, but have not yet been widely adopted in clinical practice. The ACR 20% (ACR20) improvement definition includes seven criteria. It requires ≥20% decreases in both the number of swollen and tender joints as well as ≥20% decreases in 3 or more of 5 secondary criteria (patient's global assessment of disease activity, physician's global assessment of disease activity, patient's assessment of pain, patient's self-assessed disability (e.g. HAQ score), and levels of ESR or serum CRP). These criteria have been expanded to include criteria for 50% (ACR50) and 70% (ACR70) improvement measures. Although 20% improvement represents a real, measurable response to treatment, patients with 20% improvement may still have considerable disease activity and could experience an additional 20% improvement (from a new baseline) several times before achieving clinically acceptable control of their RA. The European League Against Rheumatism (EULAR) has also developed criteria for defining improvement in RA patients. "The Disease Activity Score" (DAS) (Van der Heijde et al. 1990, 1992) uses 3 variables: RAI, the number of swollen joints and ESR which are included in a formula $DAS = 0.54 \sqrt{RAI} + 0.065$ (number of swollen joints) + 0.33 (ln ESR) + 0.224. A DAS decrease of ≥1.2 corresponds to improvement (Van Gestel et al. 1996).

Harvey et al. (1998) found that RA patients with a ACR20 response during DMARD therapy (methotrexate alone, sulfasalazine and hydroxychloroquine in combination, or all three drugs in combination) had a decrease in serum YKL-40 of 21% compared to baseline levels. Moderate responders had a decrease in serum YKL-40 of 13% and non-responders an increase in serum YKL-40 of 13%. The percentages of patients with a decrease in serum YKL-40 of ≥20% were 49% and 46% after 3 and 12 months of MTX therapy, 50% and 79% after 3 and 12 months of sulphasalazine therapy, 35% and 32% after 3 and 12 months of leflunomide, and 35% and 43% after 3 and 12 months of penicillamine therapy (Johansen et al. 2001a). Only few patients had a decrease of ≥50% in serum YKL-40 after 3 and 12 months of DMARD therapy.

Complete remission of disease activity in RA patients is defined as the absence of symptoms of active inflammatory joint pain, morning stiffness, fatigue, synovitis on joint examination, progression on radiographic damage on sequential radiographs, and elevation of ESR or serum CRP (Pinals et al. 1981). Only one study has measured serum YKL-40 levels in patients who went into remission after DMARD therapy. These patients had a significant decrease in serum YKL-40 of 30% compared to the level when the patients had active disease (Johansen et al. 1999b IV).

RA patients who favorably respond to MTX treatment have a risk of a disease flare within the first year after discontinuation of MTX treatment (Gøtzsche et al. 1996; ten Wolde et al. 1996; Sander et al. 1999). Patients with elevated serum YKL-40 at time of stopping MTX treatment were at risk of developing a relapse, and serum YKL-40 increased significantly one month before the relapse and at time of the relapse. If the patients later were treated with MTX a significant decrease in serum YKL-40 was observed after 2 months of MTX treatment (Johansen et al. 1996b; Hansen et al. unpublished).

The mechanisms by which DMARDs may have an effect on serum YKL-40 are unknown, but is likely due to a reduction of activated monocytes/macrophages and leukocytes. MTX, sulfasalazine, and penicillamine suppress inflammatory disease activity and produce alterations in synovial tissue morphology by a reduction in mononuclear cell infiltration and endothelial cell proliferation, and decrease cytokines and MMPs. The active metabolite of leflunomide inhibits dihydroorotate dehydrogenase, a critical enzyme for de novo synthesis of pyrimidines, and regulates lymphocyte proliferation, supress IL-1, TNFα, IL-2, MMP-1, ICAM-1, VCAM-1 and NFκB synthesis, increase TGFβ synthesis and inhibits adherence of leukocytes to the vascular endothelium expressions (Walters et al. 1987; Firestein et al. 1994; Cao et al. 1996; Cronstein 1996; Dolhain et al. 1998; Violin et al. 1999; Breedveld et al. 2000; Kraan et al. 2000). There are no *in vitro* studies of YKL-40 mRNA and protein expression by inflammatory cells, synovial fibroblast like cells and endothelial cells after treatment with these DMARDs.

Glucocorticoids are often used in combination with DMARDs for treatment of RA patients. Low doses of prednisolone (2.5 to 7.5 mg p.o. daily) had no effect on serum YKL-40, whereas treatment of active RA patients with medium dose of prednisolone (30 mg p.o. daily) resulted in significant decreases in serum YKL-40 of 15% after one day and 33% after one month of treatment (Johansen et al. 1999 IV, 2001b). High dose of a single intra-articular glucocorticoid injection in inflammed knee joints of RA patients was followed by a significant decrease in serum YKL-40 already after one day and the level remains decreased for at least 14 days (Volck et al. 2001). If the patient later had relapse of knee joint synovitis a corresponding increase in serum YKL-40 was observed. The mechanism of action of glucocorticoids on YKL-40 expression is not known, but it is most likely indirect through glucocorticoids many and diverse molecular effects on the cells in the arthritic joint. Glucocorticoids inhibit leukocyte, monocyte and macrophage migration to sites of inflammation, are toxic to lymphocytes and inhibit macrophage function, antigen presentation, and class II molecules expression, suppress production of cytokines (e.g. TNFα, IL-1β, IL-2, IL-6), chemokines (e.g. IL-8, RANTES, MCP-1), MMPs and TIMP-1, increase transcription of anti-inflammatory genes (e.g. IL-10 and IL-1 receptor antagonist), inhibit adhesion molecules and interferon-gamma production, induce apoptosis, inhibit angiogenesis (Hori et al. 1996), reduce cytokine-mediated E-selectin and ICAM-1 expression on endothelial and synovial lining layer cells (Firestein et al. 1991; Chikanza et al. 1993; Van den Brink et al. 1994; Pearson et al. 1995; Youssef et al. 1996, 1997; Brack et al. 1997; Barnes 1998; Kirsch et al. 1999; Moreland et al. 2002; Buttgereit et al. 2004).

Recently, new treatment modalities ("Biological treatments") for RA patients have emerged using either TNFα-blocking agents (Olsen et al. 2004) (Etanercept (Enbrel, a recombinant human TNFα receptor fused to the Fc portion of human IgG1); Infliximab (Remicade, a chimeric (75% human and 25% mouse peptide sequences) monoclonal antibody against TNFα); and Adalimumab (D2E7, human monoclonal antibody against TNFα)), a recombinant human IL-1 receptor antagonist (Anakinra) (Olsen et al. 2004) or an anti-IL-6 receptor monoclonal antibody (Choy et al. 2002). These therapies rapidly reduce clinical signs of synovitis, ESR and serum CRP in RA patients and have protective effects on cartilage and bone, and may retard or arrest radiological progression both in the early and later course of the disease. Serum YKL-40 decreased significantly in some RA patients treated for 2-4 weeks with infliximab or adalimumab, and the largest decreases were found in patients with elevated serum YKL-40 at baseline (Charles et al. 1999; den Broeder et al. 2002; Johansen et al. 2003b). The mean percentage decreases in serum YKL-40 were 20% after 1 month, 21% after 3 and 24% after 12 months of treatment. 60% and 64% of the patients had a decrease in serum YKL-40 of ≥20% after 1 and 12 months of anti-

TNFα therapy (Johansen et al. 2003b). The mechanism of action of these "Biological treatments" on YKL-40 expression is not known. There may be both a direct effect on YKL-40 gene expression and an indirect effect through the known effects by TNFα, IL-1 and IL-6 blocking agents on the cells in the arthritic joint (Choy et al. 2001).

Serum YKL-40 concentrations in RA patients in relation to radiographic joint damage

The radiographic joint damage, visible as erosions and joint space narrowing, in RA patients often begins within the first few years of the disease. 25-40% of early RA patients (i.e. < 6 months of symptoms) have bone erosions at presentation and more than 70% of RA patients develop radiographic joint damage within the first 2 years of the disease (Van der Horst-Bruinsma et al. 1998; Hulsmans et al. 2000; van der Heijde et al. 2000). Spontaneous remission of RA without treatment is rare (5-7%) (Harrison et al. 1996b; Eberhardt et al. 1998) and only 26% of treated RA patients with erosive disease at diagnosis have no radiographic progression over the next 5 years of follow-up (Fex et al. 1996). Radiographic joint damage progress linearly over time in RA patients followed in private rheumatology practice (Graudal et al. 1998; Wolfe et al. 1998; Hulsmans et al. 2000), but when looked at individual patients the radiographic progression rate of joint destruction is variable.

Several studies have demonstrated a relation between the presence of synovitis and the progression of radiological joint damage in RA patients (Scott et al. 1984, 2000; van der Heide et al. 1995; Hassell et al. 1995; Young et al. 1997; Graudal et al. 2000b; Boers et al. 2001). However, synovitis does not necessarily equate with joint damage. It has been suggested that the pathophysiologic mechanisms of joint inflammation and erosion may be partially independent. Studies have described a dissociation between clinical synovitis, serum CRP and ESR and radiological progression of joint destruction in RA patients, and despite improvement in clinical measures of synovial inflammation some patients show evidence of increased articular destruction (van Leuwen et al. 1994; Kirwan et al. 1995, 1997, 2001, 2004; Mulherin et al. 1996a, 1996b; Hickling et al. 1998; Bukhari et al. 2001). It has been hypothesized that the clinical signs and symptoms of inflammation are caused by synovial pathological processes that are different from those that cause bone erosions, and that joint space narrowing might behave differently from progression of bone erosions (Kirwan et al. 1997, 2001, 2004). Clinical determination of the number of swollen joints is not very sensitive and has a large inter-observer variability. Clinical asymptomatic joints of patients with early or chronic RA can show histological evidence of synovitis (Soden et al. 1989; Pando et al. 2000) and progression of joint destruction can occur in RA patients in prolonged clinical remission (Molenaar et al 2004). The newer imaging techniques, MRI and UL, are more objective and sensitive measures of synovial inflammation than the clinical evaluation of the number of swollen joints, and these new techniques show more sites of inflammation than joint counts of synovitis. Longitudinal MRI studies in early RA patients have demonstrated that synovitis appears to precede bone edema and subsequent erosions and that bone erosions do not occur in the absence of synovitis (McGonagle et al. 1999).

Baeten et al. (2000) found that the number of YKL-40+ cells in the synovial lining was higher in RA patients with radiological evidence of erosions than in patients without erosions. Serum YKL-40 correlated with Larsen score, the number of bone erosions or Sharp score, and RA patients with bone erosions had higher serum YKL-40 than patients without erosions (Garnero et al. 1999; Matsumoto et al. 2001; Peltoma et al. 2001; den Broeder et al. 2002). However, a single serum YKL-40 measurement in RA patients cannot predict future radiographic progression of joint damage (Johansen et al. 1999b IV, 2001b; Combe al. 2001; den Broeder et al. 2002). Two longitudinal studies of 1 or 3 years of patients with early RA have shown that the mean serum YKL-40 levels during the study periods were related with the progression in Larsen score. Patients with persistently high serum YKL-40 had larger progression in Larsen score and developed more bone erosions compared to patients with normal serum YKL-40. In the same patients a persistently elevated serum CRP was not related to progression in Larsen score during the 1 year study (Johansen et al. 1999b, IV), but in the 3 year study a high serum CRP was also related to the progression in Larsen score (Johansen et al. 2001b).

Osteoarthritis

Secondary osteoarthritis (OA) is the most prevalent disease of articular joints and is one of the major causes of disability in the elderly (Lawrance et al. 1998). OA is part of the aging process, but its etiology is far from being fully understood. There is strong evidence that the structural changes observed in OA cartilage with appearance of fibrillations, cell clusters and changes in ECM composition and with depletion of cartilage are due to combination of several different factors like biomechanical forces and aberrant behavior of resident chondrocytes (Nuki et al. 1999; Pelletier et al. 2000; Poole 2003). Cartilage degradation and loss are the major features of OA, but the disease process also affects the synovial membrane, subchondral bone, ligaments and periarticular muscles. It is unclear which factors are responsible for the initiation of OA and which stimuli regulate the chondrocyte proliferation into cell clusters and the hyperactive phenotype of OA chondrocyte. Acute joint injury or chronic exposure of cartilage to an abnormal biochemical or biomechanical environment result in activation of chondrocytes. This chondrocyte response is manifested by enhanced cell proliferation and death, ECM degradation and new matrix synthesis, and the cells synthesize large number of proinflammatory cytokines (e.g. IL-1β, TNFα, IL-6, IL-8, IL-11, IL-17), anti-inflammatory cytokines (e.g. IL-4, IL-10, IL-13), MMPs and their inhibitors, growth factors, matrix molecules and nitric oxide (a catabolic factor linked with chondrocyte apoptosis). Since chondrocytes are sequestered within lacunae of articular cartilage, a tissue that is avascular and aneural, it has been suggested that these cytokines act within cartilage in an autocrine or paracrine manner. IL-1β and TNFα are of major importance to cartilage destruction and resorption of subchondral bone in OA. They stimulate their own synthesis and induce chondrocytes, synovial macrophages and fibroblasts to produce other cytokines, leukocyte inhibitory factor, proteases, prostaglandin E2 and MMPs (MacNaul et al. 1990; Brennan et al. 1992; Lotz et al. 1995; Kammermann et al. 1996; Shlopov et al. 1997, 2000). In OA patients like in RA the MMPs (particularly MMP3), TIMPs, aggrecanase, serine- and cysteine proteinases, the plasminogen activator and its inhibitors play a role in cartilage degradation (Woessner et al. 1991; Freemont et al. 1997; Shlopov et al. 1997; Pelletier et al. 2001). As proteoglycans are lost and collagen is degraded, the articular cartilage swells. Although the chondrocytes make an attempt to repair the cartilage by increasing the production of proteoglycans at some point the damage is too great and the repair response is overwhelmed.

An association exists between synovitis and progression of structural changes of the OA joints (Pelletier et al. 2001). The presence of synovial inflammation is believed to be a secondary phenomenon related to the destruction of cartilage and the release of cartilage breakdown products into the synovial fluid. In OA synovium, the inflammatory changes that take place include synovial hypertrophy and hyperplasia with increased number of lining cells and a mixed population of inflammatory cells. Some degree of synovitis is found in early stages of OA, but in patients with later stages of OA the degree of synovitis is usually mild or moderate. In severe OA the extent of inflammation and the morphological changes in the synovial membrane can be indistinguishable from that observed in RA patients (Haraoui et al. 1991; Farahat et al. 1993; Smith et al. 1997; Nakamura et al. 1999).

The most established methods to assess progression of cartilage destruction in OA patients are the measurement of joint space width

using plain radiographs and determination of chondropathy by arthroscopy. MRI is more sensitive than radiography, but this method is not yet validated for monitoring patients with OA. There are no biomarkers available to diagnose OA at an early stage and no markers have so far gained acceptance in clinical routine for monitoring disease activity in OA patients. There are several biomarkers associated with cartilage degradation and synovial inflammation in OA such as serum levels of COMP, hyaluronan, the N-terminal propeptide of type IIA procollagen, MMPs, TIMPs and the urinary excretion of the C-terminal crosslinking telopeptide of type II collagen (Sharif et al. 1995; Conrozier et al. 1998; Petersson et al. 1998; Myers 1999; Garnero et al. 2000, 2001, 2002a; Pelletier et al. 2001; Poole 2002; Pavelka et al. 2004; Takahashi et al. 2004), and these markers are currently under investigation in longitudinal studies of OA patients. None of these biomarkers are specific for OA.

YKL-40 expression in cartilage and synovial membrane of OA patients

OA chondrocytes alter their pattern of gene expression in response to changes in their surrounding matrix, mechanical properties of the cartilage, various growth factors, cytokines, and inflammatory mediators (Buckwalter et al. 1997; Rosier et al. 1998). One of the major changes in the chondrocyte phenotype in OA involves a switch in the types of collagen molecules they synthesize. Clusters of chondrocytes in OA cartilage express type I and III collagens, which are not normally found (or at very low level) in chondrocytes of normal articular cartilage (Aigner et al. 1993, 1997; Young et al. 2000). It is suggested that OA chondrocytes express a more dedifferentiated phenotype and have a more fibroblast-like appearance than normal chondrocytes (Benya et al. 1978, 1982; Aigner et al. 1993, 1997; Stokes et al. 2002). *In vitro* the YKL-40 gene expression of dedifferentiated human fetal chondrocytes was up-regulated compared to differentiated chondrocytes (Stokes et al. 2002). *In situ* hybridization and immunohistochemical studies of OA cartilage specimens from the hip and knee joint showed YKL-40 mRNA and protein expression in chondrocytes located in the superficial and mid zone of the OA cartilage and mainly in areas of the hip joint with a considerable mechanical load (Volck et al. 1999b, 2001; Connor et al. 2000; Johansen et al. 2001c VII; Kawasaki et al. 2001). These zones are characterized by chondrocyte clusters, fibrillations, degenerative changes in ECM composition and matrix depletion, and chondrocytes in these two zones express IL-1β, TNFα, MMPs, TIMP-1, and u-PAR (Walter et al. 1998; Tetlow et al. 2001; Poole 2003). Most of the apoptotic chondrocytes are also located in the superficial zone (Hashimoto et al. 1997). No YKL-40 mRNA or protein expression were found in chondrocytes from normal cartilage (Volck et al. 1999b; Connor et al. 2000). Although YKL-40 is secreted by chondrocytes *in vitro,* immunohistochemical analysis could not detect peri- or extracellular YKL-40 protein in OA cartilage. The presence of YKL-40 in the ECM can not be excluded, since YKL-40 epitopes recognized by the antibodies may be masked by interaction with other matrix components in ECM or YKL-40 is present in a too low concentration to be detected. One study found no enhanced YKL-40 expression in OA cartilage compared to normal cartilage using cDNA array analysis and RT-PCR (Steck et al. 2002). The reason for this discrepancy with the earlier studies is unknown.

YKL-40 expression by a subpopulation of OA chondrocytes and always by clusters of chondrocytes in OA cartilage may be related to a stage specific event and suggests a role for YKL-40 such as the restructuring of the pericellular matrix surrounding the chondrocytes in OA cartilage. Chondrocytes purified from OA cartilage secreted YKL-40 *in vitro* in the absence of fetal calf serum and YKL-40 expression in degenerative cartilage may influence the capacity of chondrocytes to divide and survive. YKL-40 in physiological concentrations increased the number of chondrocytes and stimulated proteoglycan synthesis (De Ceuninck et al. 2001a) and activated MAP kinase and PI3K signaling pathways in articular chondrocytes (Recklies et al. 2002).

Immunohistochemical analysis of synovial membranes from OA patients demonstrated that some cells in the synovial lining and stroma had YKL-40 protein expression. Most of these cells were macrophages (had CD68 protein expression) (Kawasaki et al. 2001; Volck et al. 2001), but some may be fibroblast-like synovial cells. The number of YKL-40+ cells in the synovial membrane from OA patients was related with the degree of synovial inflammation and the synovial fluid concentration of YKL-40. Approximately 20% of synovial biopsies from OA patients had YKL-40+ cells, which is lower compared to synovial biopsies from RA patients where 80% had YKL-40+ cells (Volck et al. 2001). Immunohistochemical studies also showed YKL-40 protein expression in mononuclear cells in synovial membranes from patients with osteonecrosis and in pseudocapsule specimens from failed total hip arthroplasty (Kawasaki et al. 2001).

Synovial fluid and serum concentrations of YKL-40 in OA patients

YKL-40 is detectable in synovial fluid from OA patients (Table 2) (Johansen et al. 1993 I, 1996a III; Kawasaki et al. 2001; Volck et al. 2001; Schmidt-Rohlfing et al. 2002). One study found that patients with late stage OA of the knee joint had higher synovial fluid levels of YKL-40 than patients with early stage OA or traumatic knee joint disease (injured ligaments or menisci) (Johansen et al. 1996a III), and lower levels compared to RA patients (Volck et al. 2001). However two recent studies could not detect any differences in synovial fluid levels of YKL-40 between patients with OA grade 2, 3 and 4 (Kawasaki et al. 2001) or between patients with different Outerbridge and Noyes classification of the severity of cartilage degradation (Schmidt-Rohlfing et al. 2002). In patients with osteonecrosis of the hip the highest synovial fluid YKL-40 level was found in stage 3 (Kawasaki et al. 2001). YKL-40 levels in synovial fluid correlated with the MRI-determined volumes of the synovial membrane and the joint effusion (Volck et al. 2001). The highest YKL-40 levels in synovial fluid from OA joints were found in patients with moderate to severe inflammation of the synovial membrane (histological evaluation) but the level was not statistically higher compared to synovial fluid YKL-40 levels in OA joints with no or slight synovial inflammation (Volck et al. 2001). Patients with osteonecrosis of the hip or failed total hip arthroplasty had higher synovial fluid concentrations of YKL-40 compared with OA patients (Kawasaki et al. 2001). In patients with failed total hip arthroplasty YKL-40 in the synovial fluid can only originate from macrophages in the synovial membrane and neutrophils in the synovial fluid, since the cartilage tissue has been completely removed from the joint. Following autologous chondrocyte implantation the synovial fluid YKL-40 level increased 6 weeks after surgery and then decreased below the baseline levels after the cartilage repair process had ceased one year after surgery and similar results were found for the synovial fluid concentrations of MMP-1, MMP-3 and TIMP-1 (Schneider et al. 2003). Synovial fluid YKL-40 in monkeys with knee OA was also higher compared to the level in monkeys without OA (Register et al. 2001).

Approximately 10-15 fold higher YKL-40 concentrations were found in synovial fluid compared to the corresponding serum concentrations in OA patients and the concentrations were correlated (rho = 0.49-0.54) (Johansen et al. 1993 I, 1996a III; Volck et al. 2001). Eleven studies have evaluated serum YKL-40 in patients with OA (Johansen et al. 1993 I; 1996a III; Harvey et al. 1998; Maciel et al. 2000; Voss et al. 2000b; Conrozier et al. 2001; Garnero et al. 2001; Volck et al. 2001; Abe et al. 2003; Pavelka et al. 2004; Takahashi et al. 2004), and all but two (Garnero et al. 2001; Pavelka et al. 2004) found elevated serum YKL-40 in OA patients compared to healthy subjects (Table 2). Patients with late stage knee OA had higher serum YKL-40 than patients with early stage OA or traumatic joint disease (injured ligaments or menisci) who had normal serum YKL-40. The overlap between serum YKL-40 in patients with late stage

OA and controls was large and only 16%-30% of OA patients had elevated serum YKL-40 compared to controls. Patients with symptoms from several OA joints had highest serum YKL-40. Correlation between serum YKL-40 and CRP levels in OA patients was found in some (Conrozier et al. 2001; Takahashi et al. 2004) but not in all studies (Johansen et al 1996a III; Volck et al. 2001). Serum YKL-40 correlated with serum MMP-3 (Takahashi et al. 2004). No changes in serum YKL-40 were found 6 and 24 hours after knee arthroscopy of OA patients (Maciel et al. 2000). Cross sectional studies of patients with symptomatic OA showed no relations between serum YKL-40 and the radiographic joint space surface area, mean joint space width, minimal joint space width, interbone distance at the narrowest point, pain, stiffness or physical function (Conrozier et al. 2001; Garnero et al. 2001; Takahashi et al. 2004).

Ankylosing spondylitis

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease and is mostly characterized by inflammation of the sacroiliac joints, the enthesis, and the spine (Braun et al. 1998, 2000). Some patients also have peripheral arthritis. AS has a strong association with specific subtypes of HLA-B27 and may be a T-cell-driven disease (Sieper et al. 1995; Marti et al. 1999; Khan 2000), but its pathogenesis remain obscure and autoantigens are not completely defined. Patients with AS had YKL-40 expression in mononuclear cells in the synovial membrane (probably CD14+CD16+ macrophages) and in a subpopulation of PBMCs (Baeten et al. 2000). The number of mononuclear cells with YKL-40 expression in these patients was lower compared to RA patients. Three small studies have found elevated serum YKL-40 in patients with AS with severe disease activity and the changes in serum YKL-40 after treatment with infliximab correlated with changes in clinical outcome measures (Bath AS disease activity index) and serum CRP (D'Amore et al. 2000; Maksymowych et al. 2002; Johansen et al. 2003b).

Conclusions and future perspectives

YKL-40 is expressed by a subgroup of macrophages in the inflammed synovial membrane from patients with RA, OA and AS, and highest number of YKL-40+ cells are found in RA patients. The number of YKL-40+ macrophages in the synovial membrane of RA patients is related to the degree of synovial inflammation and joint erosion. YKL-40 is also expressed by fibroblast-like synovial cells from inflammed synovial membrane from RA patients. Furthermore, a subpopulation of articular chondrocytes located in the superficial and middle layer of arthritic cartilage from RA and OA patients express YKL-40 and the protein may reflect the heterogeneity of the OA chondrocyte phenotype. YKL-40 is found in mg levels in synovial fluid from RA and OA patients with severe synovial inflammation and is probably mainly derived from macrophages and fibroblast-like synovial cells in the inflammed synovial membrane and from activated neutrophils in the synovial fluid. A smaller amount of YKL-40 in the synovial fluid may originate from articular chondrocytes. The YKL-40 concentration in synovial fluid of RA and OA patients is related to the volume of the synovial membrane and to the serum concentration of YKL-40, indicating that a substantial amount of YKL-40 in serum from RA and OA patients originates from the arthritic joints.

The present studies suggest that serum YKL-40 reflects synovial inflammation in rheumatic joint diseases independent of etiology. Serum YKL-40 is elevated in approximately 40% of RA patients with clinically active disease and in 13% with mild or inactive disease compared to healthy subjects. Some but not all studies found elevated serum YKL-40 levels in OA patients compared to healthy subjects. Serum YKL-40 can not be regarded as a better biomarker than ESR and serum CRP to evaluate disease activity in RA patients, and a single measurement of serum YKL-40 can not predict the prognosis of the patients. Since YKL-40 is produced locally by cells in the arthritic joint, the assessment of serum YKL-40 may provide new and a more direct information of the local disease activity in arthritic joints compared to ESR and serum CRP. The relationship in RA patients between continuously elevated serum YKL-40 and the progression in bone erosions suggests that large prospective studies of RA patients should be performed to assess if the combined measurement of serum CRP and YKL-40 levels (and other biomarkers) are better to determine ongoing disease activity and to predict joint destruction than if only serum CRP is determined. It may also be of value to evaluate changes in serum YKL-40 (in combination with other biomarkers) in RA patients during treatment with TNFα, IL-1 and IL-6 blocking agents ("Biological treatments") since changes in these biomarkers may be useful to identify after a few months of treatment the patients who will respond to the treatment. The clinical value of serum YKL-40 as a potential biomarker in OA and AS patients is unclear, and longitudinal studies of these patients are necessary to assess the value of serum YKL-40 in monitoring disease activity and progression in patients with OA and AS.

YKL-40 is an autoantigen in RA and may play a pathogenic role in the inflammatory process and joint destruction of RA patients. Increased YKL-40 synthesis in the joints of RA patients may lead to an increased YKL-40-derived peptide presentation, which could amplify the local autoimmune response. It is not known if YKL-40 has a role in the development and progression of OA and AS. YKL-40 is a growth factor for fibroblast-like synovial cells, fibroblasts, chondrocytes, and endothelial cells. Locally secreted YKL-40 may have an autocrine and/or paracrine effect on synovial macrophages, fibroblast-like synovial cells, endothelial cells, and chondrocytes playing a role in their proliferation rate or protect them from undergoing apoptosis. The protein may also stimulates angiogensis in the synovial membrane, or exhibits a role in the pericellular ECM remodeling in RA, OA and AS patients.

If future studies show that YKL-40 has a function in the development and progression of RA then inhibition of YKL-40 expression or prevention of YKL-40 activity could offer an approach to suppress inflammation and prevent joint damage in RA patients. Such interventions include induction of immunological tolerance, inhibition of YKL-40 production (e.g. siRNA), and neutralization of YKL-40 activity (e.g. monoclonal antibodies against YKL-40 or its receptor, natural YKL-40 receptor antagonists). However, the effectiveness and toxicity of anti-YKL-40 based interventions are difficult to predict at present.

4.3. DISEASES WITH CHRONIC INFLAMMATION AND GRANULOMA FORMATION

Giant cell arteritis

Giant cell arteritis (GCA) is a systemic vasculitis that primarily affects medium-size and large arteries and is a T-cell-dependent disease. The etiology of GCA is unknown but may be an antigen driven disease, although the inciting antigen has not been identified. The activation of adventitial dendritic cells is an early event in the vasculitides. These dendritic cells secrete chemokines, which have a critical role in attracting T-cells and macrophages into the arterial wall. The inflammatory infiltrate in the arteries consist mainly of CD4+ T lymphocytes and macrophages that infiltrate all layers of the arterial wall. Plasma cells, neutrophils and B lymphocytes are sparse or absent in the inflammatory infiltrate. Granuloma formation is usually localized in the arterial media and multinucleated giant cells accumulate along the fragmented internal elastic lamina. The vasculitis causes arterial wall destruction with aneurysm formation and risk of rupture, proliferation of smooth muscle cells and myofibroblasts in the arterial intima with thickening of the intima that leads to occlusion of the arterial lumen and tissue ischemia. The proliferation of the intima is induced by growth factors (e.g. VEGF and PDGF) produced by giant cells and macrophages in close vicinity of the fragmented elastic laminae. Macrophages in the inflammatory infiltrates also secrete proinflammatory cytokines (e.g. IL-1β, IL-6, TGFβ),

MMPs and contribute to cellular damage through lipid peroxidation and nitric oxide synthase 2 expression. The T cells synthesize IL-2 and interferon-γ (IFNγ). It is not known what causes the membrane to fragment initially and to subsequently initiate production of growth factors. Systemic activation of monocytes are found in GCA and it has been suggested that GCA has two components, an inflammatory reaction in vessel walls and a systemic activation of monocytes. In patients with polymyalgia rheumatica (PMR) the systemic inflammation is the major manifestation of the disease, with vascular inflammation being maintained at a subclinical level (Weyand et al. 2003a, 2003b).

It is unknown if YKL-40 is secreted by circulating monocytes from patients with GCA or PMR, but immunohistochemical analysis of temporal artery biopsies with histological signs of GCA has shown YKL-40 protein expression in multinucleated giant cells and mononuclear cells. YKL-40 expression was detected in a subset of macrophages located in areas with granulomatous inflammation at the intima-medial junction, particularly along the internal elastic membrane (Johansen et al. 1999a V). No YKL-40 expression was found in macrophages located in the intima and adventitia or in the ECM. Temporal arteries from patients with PMR have no signs of inflammation and no cells with YKL-40 protein expression. These results indicate that YKL-40 is secreted by activated macrophages at a special stage. A close relationship exists between the localization and the function of macrophages, defined on their product profile, and at least three types of macrophages can be distinguished in the lesions of GCA patients. The localization of macrophages in the blood vessel wall may be a predictor for the functional status of the macrophages, and the microenvironment of the artery may be directly involved in regulating the function of macrophages. YKL-40 expression was only found in macrophages and giant cells located in the media and intima-medial junction of the inflammed arteries. These cells have a unique product profile in that they produce MMPs, growth factors, and angiogenic factors, but not IL-1β, IL-6 and TGFβ which are produced by the macrophages homing to the adventitia (Weyand et al. 1999). Macrophages in the media and intima-medial junction are assumed to play a role in the fragmentation and destruction of the media layer and of the elastic tissue and in mediating the arterial injury response. The function of YKL-40 in GCA is unknown, but YKL-40 is an adhesion and migration factor for vascular smooth muscle cells (Nishikawa et al. 2003) suggesting that YKL-40 could have a role in the processes which lead to progression of occlusive vascular diseases like GCA.

The vascular inflammation in GCA is associated with an intense acute-phase response. High ESR is one of five components of the American College of Rheumatology 1990 criteria for the classification of GCA (Hunder et al. 1990), and ESR and serum CRP levels are used as biomarkers of disease activity in patients with GCA and PMR. However, some GCA patients have normal ESR levels at time of diagnosis and ESR provides limited information about disease reactivation in patients on a tapering regimen of prednisolone. There has been a search for new biomarkers of disease activity in patients with GCA and PMR, and at time of diagnosis patients with GCA more often had elevated plasma IL-6 than elevated ESR. Furthermore, fewer disease relapses were missed using plasma IL-6 compared to ESR, and plasma IL-6 showed prompt responsiveness to corticosteroid therapy (Weyand et al. 2000b, 2003a).

At time of diagnosis 53% of patients with GCA and 38% with PMR had elevated serum YKL-40 compared to healthy subjects (Johansen et al. 1999a V). Serum YKL-40 was significantly elevated in patients with GCA but not in PMR patients compared to controls (Table 1). During treatment of GCA patients with high doses of prednisolone serum YKL-40 decreased significantly, and after one month of treatment serum YKL-40 was normal in most GCA patients. No changes in serum YKL-40 were found in PMR patients during prednisolone treatment. 56% of the GCA patients with signs of disease relapse had corresponding elevations in serum YKL-40 (Johansen et al. 1999a V). At time of diagnosis of GCA or PMR serum YKL-40 levels correlated with ESR and serum CRP but not during prednisolone treatment. Corticosteroids are effective in suppressing clinical manifestations of GCA, but do not shorten the course of the disease or eradicate vasculitis, and only transiently down-regulate proinflammatory cytokines and have marginal effects on IFNγ (Achkar et al. 1994; Brack et al. 1997). The ability of corticosteroids to prevent blindness may result from a reduction in vascular-wall edema or the disruption of the triggering of dendritic cells in the arterial adventitia (Weyand et al. 2003b). Glucocorticoids may not have a direct effect on YKL-40 expression since some patients with GCA and PMR treated with high doses of prednisolone had unchanged serum YKL-40 despite decreases in ESR and serum CRP. The study suggests that serum YKL-40 is not a useful biomarker of disease activity in patients with PMR. Large prospective studies of patients with GCA are needed to determine if serum YKL-40 can be used as a biomarker of disease activity in GCA patients and if serum YKL-40 provides useful clinical information different from that of ESR, serum CRP and IL-6.

Inflammatory bowel disease

Inflammatory bowel disease (IBD) comprises of two forms of chronic intestinal inflammation, ulcerative colitis (UC) and Crohn's disease (CD). The etiology of IBD is unknown. It may be an autoimmune disease and mucosal immune activation is likely to play a crucial role in the pathogenesis of IBD (Sartor et al. 2004). Intestinal inflammation in IBD include a mononuclear cell and neutrophil infiltrate and mucosal ulceration with remodeling of the ECM. In both acute and chronic inflamed intestines, healing of the damaged intestinal wall requires reconstruction of the tissue framework and remodeling of ECM, and fibrosis is a nonspecific response to the chronic inflammation observed in UC and CD patients. The localization and intensity of the inflammatory cell infiltrates may be the reason for the different clinical outcomes between UC and CD. Chronic inflammation of the intestinal submucosa is characteristic of UC and an increased collagen deposition and development of fibrotic changes are largely confined to the superficial layers of the inflamed UC intestine. Whereas the inflammatory infiltrate is transmural in CD resulting in an increased transmural collagen deposition and often followed by transmural fibrosis with luminal narrowing and stricture formation (Harper et al. 1987; Matthes et al. 1992; Lawrance et al. 1999, 2001). Relapses are frequent in CD, occurring in 50% of unselected cohorts of patients per year after diagnosis (Munkholm et al. 1995). Approximately 70% of CD patients will eventually require surgery due to strictures (Sacher et al. 1990). At present it is not possible to predict relapses and ongoing fibrogenesis in IBD patients.

Neutrophils and macrophages in the inflammed intestine from patients with UC and CD had YKL-40 protein expression (Vind et al., personal communication). YKL-40 or its peptide derivatives may be a target of the T-cell-mediated immune response in IBD and could play a role in the pathogenesis of IBD. Vos et al. (2000a) found proliferative responses of PBMC from IBD patients against YKL-40 peptides (259-271 and 263-275, which were predicted to bind to DRB1*0401 with the aid of a DRB1*0401 peptide-binding motif) suggesting that YKL-40 may be an autoantigen in IBD patients and that the immune response to YKL-40 could play a role in sustaining chronic inflammation. However, no correlation was found between the disease activity score in IBD patients and the T-cell responses against the YKL-40 peptides.

Vos et al (2000b) also reported in a small study of IBD patients that some had elevated plasma YKL-40 compared to healthy controls. This has been confirmed in three larger studies of IBD patients (Koutroubakis et al. 2003; Punzi et al. 2003; Vind et al. 2003) (Table 1). 29% of patients with active UC had elevated serum YKL-40 compared to controls and their median serum YKL-40 was higher compared to the level in inactive UC patients and controls. 38% of pa-

tients with active CD had elevated serum YKL-40 compared to controls and significantly higher median serum YKL-40 compared to controls but not to inactive CD patients. Significant correlations were found between serum YKL-40 and CRP levels and Simple Clinical Colitis Activity Index in UC patients whereas low correlations were seen in CD patients between serum YKL-40, CRP and Harvey-Bradshaw score (Vind et al. 2003). In another study serum YKL-40 was elevated in active CD patients compared to inactive CD patients using the Crohn's Disease Activity Index and significant correlations were found between serum YKL-40, CRP and this disease activity score (Koutroubakis et al. 2003). IBD patients with joint involvement had higher serum YKL-40 than patients without joint involvement (Punzi et al. 2003), and serum YKL-40 was suggested as a possible biomarker of arthropathy.

The subgroup of IBD patients with elevated serum YKL-40 may not only have intestinal inflammation or arthropathy. CD patients with stenotic disease had higher serum YKL-40 than patients with non-stenotic disease (Koutroubakis et al. 2003), and serum YKL-40 in IBD patients may reflect ongoing fibrogenesis, since YKL-40 is a growth factor for fibroblasts (Recklies et al. 2002). Large prospective studies of patients with IBD are needed to evaluate if serum YKL-40 is a useful biomarker in CD patients for determining the risk of developing stricture formation.

Sarcoidosis

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology characterized by the formation of noncaseating granuloma (Newman et al. 1997). Disease activity is accompanied by chronic inflammation with mononuclear cell infiltrates and granuloma formation. Even in the early stages of granuloma formation, a fibrotic response can be observed, and in some patients the fibrotic response results in substantial and irreversible organ destruction and physiologic dysfunction. Although essentially all organs of the body may be affected by sarcoidosis, the lungs are most commonly involved (Milman et al. 1990a), and some patients with chronic active pulmonary sarcoidosis succumb to respiratory failure (Milman et al. 1990b). The natural course of sarcoidosis is unpredictable in the individual patient. Many attempts have therefore been made to find biomarkers of disease activity in pulmonary sarcoidosis, which could help identify patients at risk of irreversible lung fibrosis. Angiotensin-converting enzyme (ACE) is produced by mononuclear cells and macrophages in the sarcoid granulomas (Allen et al. 1986) and determination of ACE concentrations in serum is used routinely to monitor disease activity of patients with sarcoidosis (Liberman et al. 1983; Allen 1991). However, serum ACE reflects the total granuloma mass and is not useful as a prognostic biomarker of severe irreversible organ dysfunction (Allen 1991).

Immunohistochemical analysis of lung biopsies from patients with pulmonary sarcoidosis has demonstrated that giant cells and mononuclear cells in the sarcoid lesions had YKL-40 protein expression. In a small pilot study of patients with pulmonary sarcoidosis serum YKL-40 was elevated in 63% of the patients compared to controls (Table 1), and serum YKL-40 and ACE were correlated. Patients with high serum YKL-40 had significantly lower lung diffusion capacity compared to patients with normal or slightly elevated serum YKL-40, whereas serum ACE could not discriminate between patients with low or high lung diffusion capacity (Johansen et al. 2005b). This study suggests that it needs to be evaluated in a large prospective study of patients with sarcoidosis whether the serum YKL-40 level in combination with lung function tests and other biomarkers of disease activity could be useful to monitor in order to identify at an early stage patients with sarcoidosis at high risk of irreversible lung fibrosis. It is unknown if YKL-40 is involved in the pathogenesis of sarcoidosis.

Systemic sclerosis

Systemic sclerosis (SSc) is an autoimmune disease characterized by initial inflammation followed by fibrotic changes of the skin, blood vessels and several organs. The pathogenesis of SSc is unclear, but various growth factors are involved in the fibrotic processes (Takehara 2003). The earliest cellular changes in affected lungs from patients with SSc are lymphocyte and plasma cell infiltration of the alveolar walls and increased numbers of macrophages in the alveolar spaces (Harrison et al. 1991). BAL studies of patients with SSc show that inflammatory alveolitis is usually characterized by an increase in alveolar macrophages and neutrophils (Silver et al. 1984; Owens et al. 1986). BAL fluid neutrophilia in patients with fibrosing alveolitis is indicative of disease progression and deterioration of lung function with fibrosing alveolitis (Wells et al. 1994; Witt et al. 1999). Interstitial fibrosis, vascular lesions including fibrous thickening of the intima, media hypertrophy, and perivascular fibrosis are found in SSc lungs and lead to a restrictive lung function pattern and impairment of the diffusing capacity (Weaver et al. 1968; Harrison et al. 1991).

Immunohistochemical analysis of a lung biopsy from a SSc patient with lung fibrosis showed YKL-40 protein expression in mononuclear cells in areas with inflammation but not in fibroblasts or in the ECM (Nordenbæk et al. 2005). Serum YKL-40 was elevated in 27%-35% of patients with SSc compared to controls (Table 1). Serum YKL-40 correlated with serum levels of soluble IL-2 receptor α and the procollagens PIIINP and PINP. Serum YKL-40 was significantly elevated in patient with arthritis, pulmonary fibrosis by chest X-ray, obstructive ventilatory pattern, reduced lung diffusing capacity and with skin retraction compared to patients without these findings. Furthermore SSc patients with elevated serum YKL-40 had shorter survival and died more often due to extensive interstitial or vascular fibrosing processes (e.g. pulmonary fibrosis, SSc renal crisis) than patients with normal serum YKL-40 (Montagna et al. 2003; Nordenbæk et al. 2005). It is unknown if YKL-40 plays a role in the pathogenesis of pulmonary fibrosis in patients with SSc. YKL-40 is a growth factor of fibroblasts (Recklies et al. 2002) and it is possible that YKL-40 takes part in the ECM remodelling process and development of fibrosis in patients with SSc. Large prospective studies of patients with SSc are needed to determine if patients with high serum YKL-40 have a poor prognosis and are at risk of developing severe organ fibrosis.

Heart transplantation

Cellular rejection is a major problem for heart, lung and kidney transplant patients. Biopsies are used for detecting transplant rejection, however the procedures are associated with morbidity and mortality. Noninvasive methods to determine transplant rejection are needed. A small pilot study of 25 heart transplant recipients found that serum YKL-40 was significantly higher in these patients compared to controls and related with the number of years since transplantation and of moderate- to high-grade rejection episodes (Fiore et al. 2000). Studies are needed to evaluate if macrophages in areas of transplant rejection express YKL-40 and if serum YKL-40 can be a biomarker in the follow-up of patients with organ transplants.

Conclusions and future perspectives

YKL-40 is produced by macrophages, giant cells and neutrophils in areas with inflammation in affected tissues from patients with diseases characterized by chronic inflammation and development of fibrosis as illustrated in patients with GCA, IBD, sarcoidosis and SSc. It has to be evaluated if YKL-40 is involved in the pathogenesis of these diseases. Serum YKL-40 is not useful as a biomarker for diagnosis of GCA, IBD, sarcoidosis and SSc, but the studies indicate that serum YKL-40 may be a valuable biomarker for monitoring disease activity in these patients. Relationships are found between serum YKL-40 and CRP and clinical parameters of disease activity in patients with chronic inflammation, but serum YKL-40 reflects other aspects of the inflammatory response than serum CRP does.

YKL-40 is produced locally in tissue with inflammation, unlike CRP production by hepatocytes in the liver. Serum YKL-40 and CRP levels may complement each other in the determination of disease activity in patients with chronic inflammation. YKL-40 stimulates growth of fibroblasts *in vitro* but the exact biological functions of YKL-40 in diseases with chronic inflammation, granuloma formations and development of fibrosis are unknown. Elevated serum YKL-40 in patients with IBD, sarcoidosis and SSc may reflect ongoing fibrogenesis that may lead, after a variable time, to clinical manifestations of severe organ fibrosis, like symptoms of fibrostenosis in the intestine of patients with CD and affected lung function in patients with sarcoidosis. Prospective longitudinal studies of changes in serum YKL-40 in these patients are needed to evaluate if serum YKL-40 can be used as a biomarker of ongoing fibrosis and to predict disease relapse.

4.4. LIVER FIBROSIS

Liver fibrogenesis represents the wound healing response of the liver and is a dynamic process with phases of either net matrix deposition or net matrix degradation leading respectively to progression or regression of liver fibrosis. The process leading to liver fibrosis includes three phases following liver tissue injury: acute inflammation, synthesis of collagenous and non-collagenous ECM components, and tissue remodeling (Bedossa et al. 2003). The ECM components of the fibrotic matrix are similar to those present in the normal liver, but in the fibrotic liver all ECM components are increased in quantity. ECM of the normal liver constitutes approximately 0.5% of the liver wet weight whereas the cirrhotic liver contains approximately six times more ECM than the normal liver. In normal liver the interstitial ECM is found in the portal area, around the central veins and in the liver capsule. The subendothelial space of Disse separates the hepatocytes from the sinusoidal endothelium and contains both an interstitial and a basement membrane-like ECM of low density. In the fibrotic liver collagenous components, particularly collagen types I and III, increase up to tenfold predominantly in the periportal and perisinusoidal space, and the perisinusoidal low-density ECM is transformed to a high-density ECM. The 3 cell types delimiting the space of Disse, hepatocytes, hepatic stellate cells (HSC, also known as Ito cells, lipocytes or fat-storing cells) and endothelial cells, all express ECM components. HSCs play a cardinal role in the pathogenesis of liver fibrosis and are the major producers of the fibrotic neomatrix and control ECM turnover (Friedman 1993, 1999; Li et al. 1999; Schuppan et al. 2001; Bedossa et al. 2003). HSCs are thought to be derived from mesenchymal cells. In the normal liver quiescent HSCs represent 5-8% of the total number of liver cells and are the major storage sites of vitamin A and are important sources of paracrine, autocrine, juxtacrine and chemoattractant factors that maintain homeostasis in the micro-environment of the hepatic sinusoid (Geerts 2001).

Following chronic liver injury (e.g. viral hepatitis infection, alcoholic or drug toxicity or any other factor that cause damage to hepatocytes) an inflammatory reaction in the liver is elicited. The damaged hepatocytes, their membrane components, metabolites of toxic agents, and infiltrating inflammatory cells activate the Kupffer cells, which release cytokines and growth factors (e.g. TGFβ, PDGF, TNFα) and reactive oxygen species. These factors (particularly TGFβ) stimulate HSCs, which then proliferate, differentiate, and undergo a major phenotypic transformation to a highly proliferative "myofibroblast-like" phenotype. Platelets and infiltrating leukocytes are also sources of cytokines that participate in HSC activation. The activated HSCs secrete a large amount of ECM components (e.g. type III collagen, structural glycoproteins, proteoglycans, hyaluronan), MMPs, TIMPs, and growth factors (e.g. VEGF, IGF-1, TGFβ, hepatocyte-, epidermal-, acidic fibroblast-, connective tissue- and hematopoietic growth factors) (Olaso et al. 1998; Maher et al. 1999; Friedman et al. 2000; Pinzani et al. 2000; Oh et al. 2001). An increase in the production of TIMPs occurs in the early stages after liver injury and persists throughout the time course of development of liver fibrosis. Due to relatively less MMPs production and an increased synthesis of TIMPs the overall net consequence leads to an enhanced deposition of ECM (Benyon et al. 2001; Schuppan et al. 2001). If the source of liver injury is removed a single liver tissue injury most often results in an almost complete resolution. During the recovery phase there is an increased MMP activity in the liver, a decrease in the expression of TIMPs, and increased apoptosis of activated HSCs. Regression of liver fibrosis is therefore characterized by degradation of fibrillar liver matrix and restoration of normal liver histology (Iredale et al. 1998; Iredale et al. 2001). Conversely, the persistence of the original liver toxic agent causes the prolonged activation of the tissue repair mechanisms, thus leading to liver fibrosis rather than to effective liver repair.

A liver biopsy is the key examination for the diagnosis and staging of liver fibrosis. However, the use of a liver biopsy can not be used as a general screening procedure for liver fibrosis or used repeatedly to monitor disease progression during the follow-up of patients with liver fibrosis. A liver biopsy has some discomfort for the patient, it requires hospitalization, and there is a risk of complications with a mortality of 0.015% (Piccinino et al. 1986). There is also a sampling error of approximately 24% false-negative (Nord 1982) and the liver biopsy does not reflect the dynamic of fibrous tissue turnover. Non-invasive methods to determine liver fibrosis are therefore needed both to diagnose significant liver fibrosis and to monitor effects of therapy on fibrogenesis and fibrolysis. Consequently there has for many years been a search for biomarkers of liver fibrosis. Several such markers have been proposed (e.g. hyaluronan, PIIINP, MMP-2, MMP-9 and TIMP-1) (Bentsen 1992; Oh et al. 2001), but none are yet used routinely in the clinical practice. However, it has been forested that a panel of biomarkers of liver fibrosis in the future will replace sequential liver biopsy as a standard care and that serum YKL-40 may be one of these markers (Oh et al. 2001).

YKL-40 expression in the liver

In 1993 Hakala et al. reported that YKL-40 mRNA was strongly expressed in human liver tissue, but it could not be determined whether the mRNA used in the experiments originated from normal or fibrotic liver tissue. Hu et al. (1996) was unable to demonstrate YKL-40 mRNA expression in normal liver tissue. Immunohistochemical analysis of liver biopsies from patients with different liver diseases have shown YKL-40 protein expression in areas with slight fibrosis (either pericellular or perisinusoidal), along fibrotic septa in association with signs of fibrogenesis, and in areas with moderate and severe fibrosis (Johansen et al. 1997, 2000a VI). Hepatocytes did not express YKL-40 protein and no expression was found in normal liver tissue except in mesenchymal structures of the portal tract. Patients with chronic active HCV had YKL-40 protein expression in areas with piecemeal necrosis but not in the lymphocytes. YKL-40 protein expression was found in 90% of liver biopsies with moderate or severe fibrosis, in 86% with slight fibrosis, and in 93% with fibrogenesis. YKL-40 expression was found in ECM both in areas free of cells as well as in cellular areas (Johansen et al. 2000a VI). It was not possible by the immunohistochemical method to discriminate the extent to which the YKL-40 protein expression was intracellular, and if HSCs, leukocytes and macrophages expressed YKL-40. These 3 cell types are probably responsible for YKL-40 synthesis in the fibrotic liver, and ongoing *in vitro* studies demonstrate that HSCs express YKL-40 mRNA (Eva Efsen, unpublished). YKL-40 is a growth factor for fibroblasts, works synergistically with IGF-1 (Recklies et al. 2002), and YKL-40 may play a role in the pathological conditions leading to liver fibrosis. Recently Shackel et al. (2003) showed using suppression subtractive hybridization and quantitative real-time RT-PCR that YKL-40 was one of the most differentially expressed genes in liver tissue from endstage cirrhosis due to HCV compared to non-diseased liver tissue, primary biliary cirrhosis and autoimmune hepatitis associated cirrhosis.

Serum concentrations of YKL-40 in patients with liver disease in relation to liver fibrosis, disease activity and prognosis

Haemodynamic investigations with catherization of the liver vein and femoral vein have shown that YKL-40 was released from the hepatosplanchnic area (Johansen et al. 1997). The serum concentration of YKL-40 in the hepatic vein was significantly higher than in the femoral artery both in patients with liver diseases and in subjects with normal liver function. Furthermore, the hepatic venous-arterial difference in serum YKL-40 concentration and the release rate of YKL-40 from the hepatosplanchnic area were higher in patients with liver disease compared to subjects with normal liver function.

Six studies have evaluated the serum concentrations of YKL-40 in patients with liver diseases. All found elevated serum YKL-40 in patients with liver fibrosis (defined from a liver biopsy) compared to healthy controls (Johansen et al. 1997, 2000a VI; Nunes et al. 1998; Tran et al. 2000; Nøjgaard et al. 2003a, 2003b). The highest serum YKL-40 levels were found in patients with alcoholic cirrhosis and in patients with cirrhosis caused by chronic hepatitis C or B virus (Table 1), indicating that elevations in serum YKL-40 in patients with liver fibrosis are independent of disease etiology. **Figure 3** illustrates the individual serum YKL-40 levels in patients with different liver diseases according to the degree of liver fibrosis. Highest serum YKL-40 were measured in patients with moderate and severe liver fibrosis (Johansen et al. 2000a VI). Nøjgaard et al. (2003a) demonstrated in a large group of patients with alcoholic liver disease that serum YKL-40 was useful to discriminate between the absence and presence of fibrosis but could not discriminate between the different degrees of liver fibrosis. 75% of the alcoholics with moderate or severe fibrosis had elevated serum YKL-40 compared to healthy controls whereas only 26% of the alcoholics without liver fibrosis had elevated serum YKL-40. Serum PIIINP was found useful to discriminate between the different degree of liver fibrosis. Serum YKL-40 was also increased in the presence of liver inflammation (Tran et al. 2000), and patients with liver fibrosis in combination with alcoholic hepatitis had higher serum YKL-40 compared to patients with fibrosis but without alcoholic hepatitis (Johansen et al. 2000a VI; Nøjgaard et al. 2003a). This suggests that some of the circulating YKL-40 in patients with alcoholic liver disease originate from activated neutrophils, which play a role in the pathophysiology of alcoholic hepatitis. The morphological features in alcoholic hepatitis include liver-cell damage, inflammatory cell infiltrate of predominantly neutrophils, and fibrosis (Poulsen et al. 1979; Baptista et al. 1988). Neutrophilia is frequent and the neutrophils are activated in alcoholic hepatitis and produce proinflammatory cytokines, chemokines and reactive oxygen species (Taieb et al. 2000). The role of YKL-40 secreted from the activated neutrophils in areas with alcoholic hepatitis is unknown, but it may participate in maintaining the liver inflammation, activate the HSCs and stimulate ECM production.

Serum concentrations of YKL-40 in patients with liver diseases correlated with other ECM products secreted by HSCs (serum PIIINP, hyaluronan, MMP-2 and TIMP-1). Low correlations were found between serum YKL-40 and enzymes secreted by hepatocytes (serum aspartate aminotransferase and alkaline phosphatase) and inverse correlations were found with serum albumin and the coagulation factors 2,7 and 10 (Johansen et al. 1997, 2000a VI; Tran et al. 2000; Nøjgaard et al. 2003a, 2003b). Serum YKL-40 correlated with other parameters reflecting the degree of liver fibrosis, such as hepatic venous pressure gradient and the postsinusoidal resistance and inversely with the clearance of indocyanine green (Johansen et al. 1997).

Nunes et al. (1998) found that serum YKL-40 decreased in patients with chronic HCV who responded to interferon treatment and Nøjgaard et al. (2003b) found that patients with chronic HCV treated for 12 months with alpha-interferon and ribavirin had a decrease in serum YKL-40 at 6 months after the end of treatment. In the patients who responded to treatment, serum YKL-40 was not related to changes in HCV titer or the liver enzymes during 12 months of treatment. The serum YKL-40 level before therapy could not predict whether a patient would respond to treatment, but the non-responders had unchanged high serum YKL-40 during the 12 months treatment period and at 6 months after therapy.

Nøjgaard et al. (2003a) reported that patients with alcohol induced liver disease and high serum YKL-40 had shorter survival than alcoholics with normal serum YKL-40 (Relative risk = 4.24, 95% confidence interval 2.18-8.26, $p<0.0001$). Multivariate Cox regression analysis including serum YKL-40 and variables known to have prognostic information of survival in alcoholics (i.e. years of high alcohol intake, serum creatinine, coagulation factors 2,7, and 10, alkaline phosphatase and IgM) showed that serum YKL-40 had no independent prognostic value.

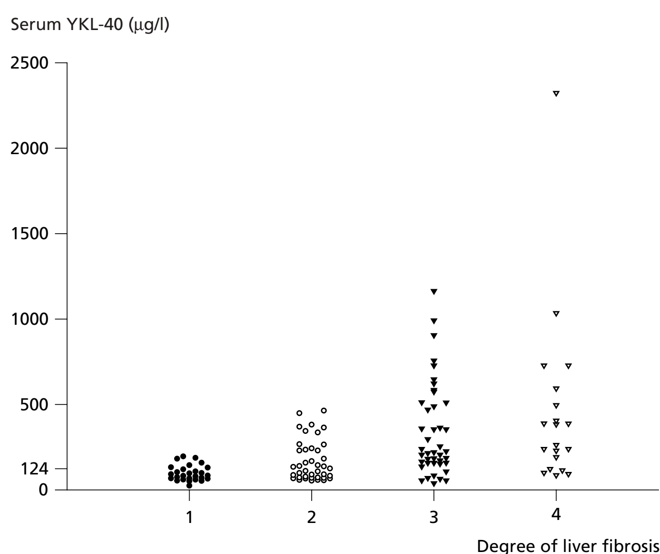

Figure 3. Individual serum YKL-40 concentrations in patients with different degree of liver fibrosis (Johansen et al. 2000a VI). 1 = no fibrosis, 2 = slight fibrosis, 3 = moderate fibrosis, 4 = severe fibrosis. The serum YKL-40 levels were determined by RIA (Johansen et al. 1993 I) but the data was corrected to ELISA values (YKL-40 ELISA=YKL-40 RIA X 0.479). The upper 95th percent limit of serum YKL-40 in 245 healthy adults is 124 μg/l.

Conclusions and future perspectives

Increased YKL-40 mRNA and protein expressions are found in fibrotic liver tissue from patients with alcoholic liver disease and chronic HCV infection. Serum concentrations of YKL-40 are elevated in most patients with moderate to severe liver fibrosis and cirrhosis, independently of disease etiology, and may provide new information of ongoing fibrogenesis in the liver. Patients with alcoholic liver disease and high serum YKL-40 have a poorer prognosis compared to patients with normal serum YKL-40. Large prospective studies of patients with liver diseases are needed to determine if patients with slight liver fibrosis and high serum YKL-40 are at risk of developing cirrhosis, and if serum YKL-40 in combination with other biomarkers of liver fibrosis (e.g. serum hyaluronan and PIIINP) can predict the severity of liver fibrosis and be used in monitoring patients with liver fibrosis or cirrhosis. Serum YKL-40 may also be useful to monitor in patients with liver diseases during anti-fibrotic or anti-viral therapy. The biological function of YKL-40 in liver diseases is not known and it needs to be determined if YKL-40 has a role in the pathogenesis of liver cirrhosis. As has been found for fibroblasts YKL-40 may be a growth factor for HSCs and could stimulate their production of collagen. Reducing the ECM production by activated HSCs is crucial in preventing liver fibrosis. If YKL-40 has a role in development of liver fibrosis then inhibition of YKL-40 production or blocking of YKL-40 activity in patients with alcoholic liver disease or hepatitis C or B virus may be a valuable method to inhibit the development of liver fibrosis.

5. YKL-40 IN CANCER DISEASES

In a search of new bone proteins it was discovered more than 10 years ago that YKL-40 was secreted *in vitro* in large amount by a human osteosarcoma cell line MG63 (Johansen et al. 1992). Two years later Morrison et al. (1994) reported that YKL-40 mRNA was expressed by murine mammary tumors initiated by neu/ras oncogenes but not by c-myc or int-2 oncogenes. Although MG63 cells originate from an osteosarcoma, these cells also have chondrocyte characteristics, since their metastases mainly consist of proliferating nodules of hypercellular cartilage (Heremans et al. 1978). Furthermore, unstimulated MG63 cells synthesize larger amount of type III collagen than of type I collagen and secrete low to undetectable levels of alkaline phosphatase and osteocalcin (Franceschi et al. 1988). Today it is known that many different types of human solid cancer express YKL-40. A search of the YKL-40 sequence against the *dbest* database at the National Center for Biotechnology Information showed that several types of solid cancer (breast-, colon-, lung-, kidney-, pancreas-, ovarian-, prostate-, and uterine carcinoma, osteosarcoma, oligodendroglioma, glioblastoma and germ cell tumors) overexpressed YKL-40. Microarray gene analyses have identified the YKL-40 gene to be one of the most highly over-expressed genes in high-grade malignant gliomas (Lal et al. 1999; Markert et al. 2001; Tanwar et al. 2002), in papillary thyroid carcinoma (Huang et al. 2001), and in extracellular myxoid chondrosarcoma (Sjögren et al. 2003). YKL-40 is not expressed by myxoid liposarcomas (Sjögren et al. 2003). Shostak et al. (2003) used public databases of the Cancer Genome Anatomy Project and found enhanced expression of the YKL-40 gene in glioblastoma multiforme and occasionally in anaplastic astrocytomas compared to normal brain. The upregulation of YKL-40 in glioblastoma multiforma is also confirmed on the protein level by Western blotting where 65% of the investigated glioblastoma multiforma samples had stronger YKL-40 protein expression than low-grade gliomas (Tanwar et al. 2002). YKL-40 is also secreted *in vitro* by human glioblastoma cells (Junker et al. 2005b) and by the monocyte-like human histiocytic lymphoma cell line U937 (Verhoeckx et al. 2004).

The biological function of YKL-40 in cancer diseases is unknown. It has been hypothesized that YKL-40 is a growth factor of cancer cells or protects them from undergoing apoptosis. YKL-40 is also called the "breast regression protein (Brp-39)" (Morrison et al. 1994) because it is induced in mice mammary epithelial cells a few days after weaning. Mammary involution involves programmed cell death, and it has been hypothesized that YKL-40 utilizes a chitin oligosaccharide binding ability while participating in various signal transduction pathways leading to apoptosis of regressing cells, and that YKL-40 is a protective signaling factor determining which cells are to survive the drastic tissue remodeling that occurs during involution (Mohanty et al. 2003). Cancer cells that express YKL-40 may have a different phenotype compared to cancer cells without YKL-40 expression, and the protein may reflect differences in the biology of various cancer cells. Neoplasms are biologically heterogeneous and contain subpopulations of cancer cells with different angiogenic, invasive and metastatic properties. Metastases can have a clonal origin, and different metastases can originate from the proliferation of different single cells. The process of metastases is selective for cells that succeed in promoting angiogenesis, invasion, embolisation, survival in the circulation, arrest in distant capillary beds and extravasation into and multiplication within the organ parenchyma. It need to be determined if YKL-40 has a role in one of these processes.

Cancer progression depends on the interplay between the cancer cells and their micro-environment, particularly the surrounding ECM, and the balance between synthesis and degradation of ECM components is a key modulator of cancer growth and metastasis (Boudreau et al. 1998). The stroma around the periphery of solid cancers have several similarities with granulation tissue such as that found in wound-healing or inflammation (Dvorak 1986; Gregoire et al. 1995), and tumors are called "wounds that never heal" (Balkwill et al. 2001). Recent studies have shown that tumor-associated macrophages and leukocytes play important roles in tumor growth and metastasis, since these cells produce growth and angiogenic factors, chemokines, chemotactic factors, MMPs and other ECM degrading enzymes (Sunderkötter et al. 1994; Lin et al. 2001, 2004ab; Bingle et al. 2002; Pollard 2004). Ongoing immunohistochemical analysis of YKL-40 expression in biopsies from breast cancer (Anne Roslind; manuscript in preparation) and colorectal cancer (personal observation) show that cancer cells in some biopsies have YKL-40 protein expression. Furthermore macrophages and neutrophils in the stroma surrounding the breast and colorectal cancer cells have YKL-40 protein expression. *In situ* hybridization of YKL-40 mRNA expression in biopsies from small cell lung cancer shows no YKL-40 mRNA expression in the cancer cells but strong expression in peritumoral macrophages (Junker et al. 2005a). It is unknown if these macrophages are CD14+,CD16+, a phenotype that express YKL-40 in RA patients (Baeten et al. 2000) and is increased in number in patients with solid cancers (Saleh et al. 1995).

YKL-40 purified from the MG63 osteosarcoma cell line has growth factor activity for fibroblast cell lines (Recklies et al. 2002). One could speculate that YKL-40 secreted by cancer cells and tumor-associated macrophages and neutrophils has a role in proliferation, activation and differentiation of the fibroblasts/myofibroblasts surrounding the tumor, and thereby influence development of the prominent desmoplastic fibroblast stroma seen in both primary cancer and metastatic sites. The phenomenon, termed stromal reaction, includes activation of fibroblast or myofibroblastic transformation, enhanced secretion of matrix proteins and MMPs, and neovascularization all of which promote proliferation, differentiation, invasion or regression of cancer cells and destruction of the stroma (Dvorak 1986; Basset et al. 1990; Gregoire et al. 1995; Rønnov-Jessen et al. 1996; Bissell et al. 2001; Kenny et al. 2003).

YKL-40 also stimulates migration of endothelial cells at a level comparable to that achieved by bFGF (Malinda et al. 1999) and modulates vascular endothelial cell morphology by promoting the formation of branching tubules. YKL-40 may therefore be a positive regulator of angiogenesis surrounding the tumor and could play a role in the growth of primary and metastatic tumors. Junker et al. (2005b) found upregulated YKL-40 expression in a human glioblastoma cell line by genotoxic and micro-environmental stress (i.e. exposure to hypoxia, ionizing radiation, etoposide, ceramide, p53 inhibition, antioxidant treatment, confluence, and serum depletion). The response in YKL-40 expression was late, 24-72 hours after stimuli, indicating that YKL-40 is a secondary response downstream of other mechanisms.

One can therefore speculate that YKL-40 is involved in proliferation of cancer cells, the surrounding tissue remodeling processes and angiogenesis, and that serum concentrations of YKL-40 may be a novel "Tumor marker". The term "Tumor marker" embraces a spectrum of molecules of widely divergent characteristics (e.g. cytogenic markers, oncogenes and abnormally expressed proteins with various biological functions), but sharing an association with malignancy that facilitates their application in the clinical detection (diagnosis, screening) and management (monitoring, prognosis) of cancer patients. Tumor markers are biological compounds, produced either by tumor cells or by the host in response to a developing tumor and are usually determined in serum. A large number of proteins have been suggested as potential circulating "Tumor markers" (Sturgeon 2002): e.g. 1) serum carcinoembryonic antigen (CEA) (Hayes et al. 1996; Mitchell 1998; McLeod et al. 1999; Compton et al. 2000; Thomas et al. 2001; Duffy et al. 2003) and plasma TIMP-1 (Holten-Andersen et al. 2000, 2002; Duffy et al. 2003) in colorectal cancer; 2) serum CA-125 (Bast et al. 1998, 2003) and tetranectin (Høgdall et al. 2000a) in ovarian cancer; 3) serum prostate specific antigen (PSA) in prostate cancer (Catalona 1994; Partin et al. 1997; Canto et al. 2004; Hittelman et al. 2004; Khan et al. 2004); 4) serum

alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG) in testis cancer (Bosl et al. 1997); and 5) serum breast cancer associated antigen 549 and 15.3 (Söletormos 2001), plasma soluble urokinase plasminogen activator receptor (Riisbro et al. 2002) and serum human epidermal growth factor receptor 2 (HER-2) in breast cancer (Ross et al. 1998; Carney WP 2003). None of these biomarkers are specific for cancer and are not yet used routinely in screening for cancer. Serum CEA, CA-125, PSA, AFP and hCG are applied routinely in patients suspected of having cancer and in monitoring of cancer patients.

IS SERUM YKL-40 A NEW BIOMARKER IN CANCER PATIENTS?

Acceptance of novel tumor markers in clinical settings requires thorough validation before being implemented into routine clinical use. Werner et al. (1993) have suggested that "Tumor markers" are classified according to six different clinical criteria such as biochemical characteristics, organ specificity or clinical usefulness in order to assess the value of tumor markers in clinical practice:

1. "The marker is produced exclusively by specific tumor cells (tumor specific)"?

YKL-40 is not specific for cancer or a certain type of tumors. YKL-40 is produced by non-malignant cells (as described in Chapter 1 and 4) and by cancer cells of widely different types of solid cancer.

2. "The marker is absent in healthy or benign disease (high specificity)"?

Serum concentrations of YKL-40 do not have high specificity for cancer. YKL-40 is detected in serum from healthy subjects and elevated serum YKL-40 (compared to healthy subjects) are found in patients with non-malignant diseases such as severe bacterial infections, active RA, GCA, IBD, lung sarcoidosis, and liver fibrosis (as described in Chapter 4). Most patients with these diseases will have some clinical symptoms of their disease.

3. "The marker is present frequently in the targeted malignancy (high sensitivity)"?

In 1995 Johansen et al. (II) reported that some patients with metastatic breast cancer had increased serum YKL-40 compared to healthy subjects, and that the highest serum YKL-40 were found in patients with short survival (**Figure 4**). This study suggested that serum YKL-40 might be useful as a prognostic marker in breast cancer patients. Recent studies have found elevated serum YKL-40 in a subgroup of patients with seven different types of localized or metastatic solid cancer compared to healthy subjects (**Table 3**) (Cintin et al. 1999, 2002; Tanwar et al. 2002; Dehn et al. 2003; Geertsen et al. 2003; Høgdall et al. 2003; Jensen et al. 2003; Johansen et al. 2003a, 2004; Dupont et al. 2004; Brasso et al. 2006). It needs to be determined if YKL-40 is elevated in serum of patients with hematological malignancies.

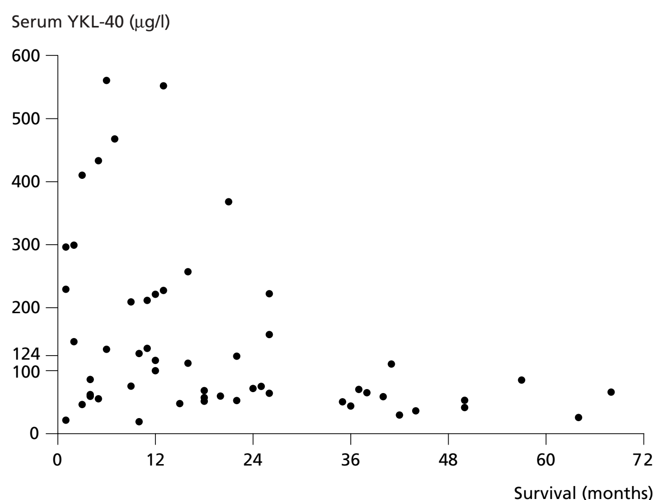

Figure 4. Individual serum YKL-40 concentrations in patients with metastatic breast cancer in relation to months of survival after the serum sample was obtained (Johansen et al. 1995 II). The serum YKL-40 levels were determined by RIA (Johansen et al. 1993 I) but the data were corrected to ELISA values (YKL-40 ELISA=YKL-40 RIA X 0.479). The upper 95th percent limit of serum YKL-40 in 245 healthy adults is 124 μg/l.

Preoperative serum YKL-40 levels were elevated in 19% of patients with primary breast cancer and the patients with metastases to axillary lymph nodes had higher serum YKL-40 compared to lymph node negative patients (Johansen et al. 2003a). In patients with first recurrence of breast cancer serum YKL-40 was elevated in 31-41% of the patients, and high serum YKL-40 was associated with metastatic sites and large tumor load: 9-20% of patients with recurrence to lymph nodes or skin only had elevated serum YKL-40, 24-35% with bone metastases, and 57-61% with visceral metastases (Johansen et al. 1995; Jensen et al. 2003). Highest serum YKL-40 were found in patients with more than two different metastatic sites (Jensen et al. 2003). Preoperative serum levels of YKL-40 from patients with colorectal cancer was elevated in 26% and there was an association between serum YKL-40 and Dukes' stage: 16% of the patients with Dukes' A (tumor confined within the bowel wall, no lymph-node metastases), 26% with Dukes' B (tumor extending through the bowel wall, no lymph-node metastases), 19% with Dukes' C (regional lymph-node metastases), and 39% with Dukes' D disseminated disease) had elevated preoperative serum YKL-40 (Cintin et al. 1999). Serum YKL-40 decreased significantly after curative operation for colorectal cancer in patients with high preoperative serum YKL-40 (Cintin et al. 2002), indicating that serum YKL-40 reflect tumor burden. Preoperative serum YKL-40 was elevated in 65% of stage I and II ovarian cancer patients (Dupont et al. 2004), in 74-91% of patients with ovarian cancer stage III (tumor growth involving one or both ovaries with wide-spread intraperitoneal metastases) and IV (disseminated disease) (Høgdall et al. 2003; Dupont et al. 2004) and in 55% of ovarian cancer patients at time of first recurrence (Dehn et al. 2003). In patients with small cell lung cancer 22% with local disease and 40% with extended disease had elevated serum YKL-40 (Johansen et al. 2004). 43% of patients with metastatic prostate cancer (Brasso et al. 2006) and 83% of patients with metastatic renal cell cancer (Geertsen et al. 2003) had elevated serum YKL-40. In patients with glioblastoma serum YKL-40 was related to tumor grade and burden: 72% of patients with glioblastoma multiforme and 57% with lower grade gliomas had high serum YKL-40 (Tanwar et al. 2002).

These studies demonstrate that serum YKL-40 does not have a high sensitivity for solid carcinoma, suggesting that not all tumors express YKL-40 or it is secreted at a low level. This could be evaluated in immunohistochemical and *in situ* hybridization studies of biopsies from different types of cancer.

4. "The marker is detectable in early stage subclinical disease (useful for screening)"?

Determination of serum YKL-40 concentrations cannot be used as a single screening test for cancer. At time of first cancer diagnosis 16-74% of the patients had elevated serum YKL-40, and only 16-26% of patients with primary localized cancer had elevated serum YKL-40. However, in patients with locally advanced or metastatic cancer at the time of diagnosis serum YKL-40 levels were elevated in 39-83%. A high serum YKL-40 in a subject without any known disease may therefore indicate non-symptomatic cancer. Serum concentrations of YKL-40 were independent of serum CEA in colorectal cancer patients (Cintin et al. 1999, 2002), of serum CA-125 in ovarian cancer patients (Dehn et al. 2003; Høgdall et al. 2003), of serum HER-2 in metastatic breast cancer patients (Jensen et al. 2003), of serum LDH in patients with small cell lung cancer (Johansen et al. 2004) and of serum PSA in patients with metastatic prostate cancer (Brasso et al.

Table 3. Serum levels of YKL-40 (μg/l) in patients with localized or advanced cancer and the percentage of patients with elevated serum YKL-40.

Diagnosis	N	Serum YKL-40		High YKL-40 (%)#	Reference
Primary breast cancer¤	271	57[c]	(22-688)	19	Johansen et al. 2003a
Metastatic breast cancer§, relapse	54	80[c]	(20-560)	41	Johansen et al. 1995
soft tissue	10	59	(29-433)	20	
bone	25	75[c]	(21-560)	35	
viscera	19	157[c]	(20-468)	61	
Metastatic breast cancer, 1. relapse	100	65[c]	(20-430)	31	Jensen et al. 2003
nodes and skin only	36	51	(20-267)	9	
bone	28	61[c]	(24-310)	24	
viscera	36	110[c]	(21-430)	57	
Colorectal cancer ¤§	603	86[c]	(27-1298)	26	Cintin et al. 1999
Dukes A	58	73[b]	(27-295)	16	
Dukes B	223	86[c]	(27-604)	26	
Dukes C	175	77[c]	(27-582)	19	
Dukes D	147	119[c]	(27-1298)	39	
Glioblastoma multiforme	45	130[c]	(38-654)	72	Tanwar et al. 2002
Lower grade gliomas	20	101[c]	(50-225)	57	
Ovarian cancer, all stages¤	50	94[c]	(17-517)	72	Dupont et al. 2004
Ovarian cancer, stage III¤	47	168[c]	(32-1808)	74	Høgdall et al. 2003
Ovarian cancer, relapse	73	94[c]	(20-1970)	55	Dehn et al. 2003
Small cell lung cancer§	131	82[c]	(23-1188)	32	Johansen al. 2004
local disease	59	71[a]	(23-417)	22	
extensive disease	72	101[c]	(27-1188)	40	
Metastatic prostate cancer	153	112[c]	(20-2080)	43	Brasso et al. 2006
Metastatic renal cell cancer	58	235[c]	(45-1896)	83	Geertsen et al. 2003

Values are median (range).

a: $p<0.02$, b: $p<0.01$ and c: $p<0.001$, compared with controls (Mann-Whitney test).

¤) Preoperative.

#) The percentage (%) of patients with elevated serum YKL-40 compared to the age-adjusted serum YKL-40 level in healthy subjects. For all the danish studies the normal reference region was calculated on the log transformed serum or plasma YKL-40 levels obtained from healthy subjects (aged 18-79 years; N=260 for RIA values and N=245 for ELISA values) (Johansen et al. 1996a III). The upper 95th per cent confidence limit was chosen for the limit and adjusted for age (Royston 1991).

§) RIA analysis (Johansen et al. 1993 I) but data corrected to ELISA values (YKL-40 ELISA=YKL-40 RIA X 0.479). All the other studies used the ELISA method (Harvey et al. 1998).

Table 4. Serum level of YKL-40 is an independent prognostic variable of overall survival in cancer patients. These results are from multivariate Cox regression analysis using routinely used prognostic variables.

Diagnosis	Relative hazard ratio	95% confidence interval	p value	Reference
Primary breast cancer	1.8	1.0-3.1	0.04	Johansen et al. 2003a
Metastatic breast cancer	2.6	1.6-4.1	0.0002	Jensen et al. 2003
Colorectal cancer	1.4	1.1-1.8	0.007	Cintin et al. 1999
Ovarian cancer stage III	4.0	1.5-10.3	0.005	Høgdall et al. 2003
Recurrent ovarian cancer	2.3	1.3-4.1	0.006	Dehn et al. 2003
Small cell lung cancer	1.9	1.1-3.4	0.02	Johansen et al. 2004
Metastatic prostate cancer	1.3	1.0-1.7	0.02	Brasso et al. 2006
Metastatic renal cell cancer	4.1	1.9-8.8	0.001	Geertsen et al. 2003

These cancer patients were scored as having elevated serum YKL-40 if it was higher than the upper 95th per cent confidence limit of serum YKL-40 in healthy subjects adjusted for age (se also footnotes to Table 3).

2006). The studies indicate that serum YKL-40 reflects other aspects of tumor growth and metastasis than these tumor markers. It may be of value to include serum YKL-40 as a biomarker for screening of cancer together with a panel of other tumor markers and imaging techniques, since an elevated serum YKL-40 level seems to reflect metastatic disease and secretion from a subset of tumors with a more aggressive phenotype and with a poor prognosis (described below).

5. "The marker's concentration reflects prognosis for an individual patient (prognosticator)"?

Eight studies have demonstrated that elevated serum YKL-40 in patients with breast-, colorectal-, ovarian-, kidney-, small cell lung-, and prostate carcinomas was an independent prognostic parameter of short recurrence free interval and short overall survival with hazard ratios between 1.3 and 4.1 (**Table 4**). This observation was found in patients with local or metastatic cancer, and at the time of first cancer diagnosis or at the time of relapse (Johansen et al. 1995, 2003a, 2004; Cintin et al. 1999, 2002; Dehn et al. 2003; Geertsen et al. 2003; Høgdall et al. 2003; Jensen et al. 2003; Brasso et al. 2006).

High preoperative serum YKL-40 level in patients with primary breast cancer was an independent prognostic parameter of short recurrence free interval and short overall survival when axillary lymph node- and estrogen receptor status, age, tumor size and histology, menopausal status and serum YKL-40 were included in the multivariate Cox analysis (Johansen et al. 2003a). There are no longitudinal studies of the changes in serum YKL-40 levels in breast cancer patients after operation and adjuvant chemo-, antiestrogen- or radio-therapy. However, an elevated serum YKL-40 in breast cancer patients at time of first recurrence predicted shorter time to progression and shorter overall survival (Jensen et al. 2003). Multivariate Cox analysis (including estrogen receptor- and axillary lymph node status at primary diagnosis, liver metastases, more than two metastatic sites, symptomatic disease at recurrence and serum HER-2

and YKL-40 levels) showed that high serum levels of YKL-40 and HER-2 were independent prognostic variables of short time to disease progression and death (Jensen et al. 2003). **Figure 5** illustrates survival curves in patients with metastatic breast cancer according to elevated or normal serum concentrations of YKL-40 and HER-2 at time of first relapse (Jensen et al. 2003). Patients with both high serum YKL-40 and HER-2 level had the poorest median survival of only 9 months contrasting 32 months for patients with normal serum YKL-40 and HER-2.

High preoperative serum concentration of YKL-40 in patients with colorectal cancer was also an independent prognostic parameter of short recurrence free interval and short overall survival (the multivariate Cox analysis included Dukes' stage, age, gender, serum CEA and YKL-40) (Cintin et al. 1999). In stage III ovarian cancer patients a high preoperative serum YKL-40 was an independent prognostic parameter of short survival (the multivariate Cox analysis included serum YKL-40 and CA-125, optimal vs. suboptimal results from primary surgery, age, and histological type of tumor) (Høgdall et al. 2003) and a similar result was found in patients with recurrence of ovarian cancer (the multivariate Cox analysis included serum YKL-40 and CA-125, age, localization of tumor and its size, performance status, primary and second-line treatment) (Dehn et al. 2003). An elevated serum YKL-40 was also an independent prognostic variable of short survival in patients with metastatic prostate cancer (the multivariate Cox analysis included age, performance status, tumor grade, serum PSA, total and bone alkaline phosphatase, PINP, crosslaps and YKL-40) (Brasso et al. 2006), and in patients with metastatic renal cell carcinoma (the multivariate Cox analysis included serum YKL-40, performance status, number of organ disease sites, organ site involvement, prior nephrectomy, and time from diagnosis to metastases) (Geertsen et al.

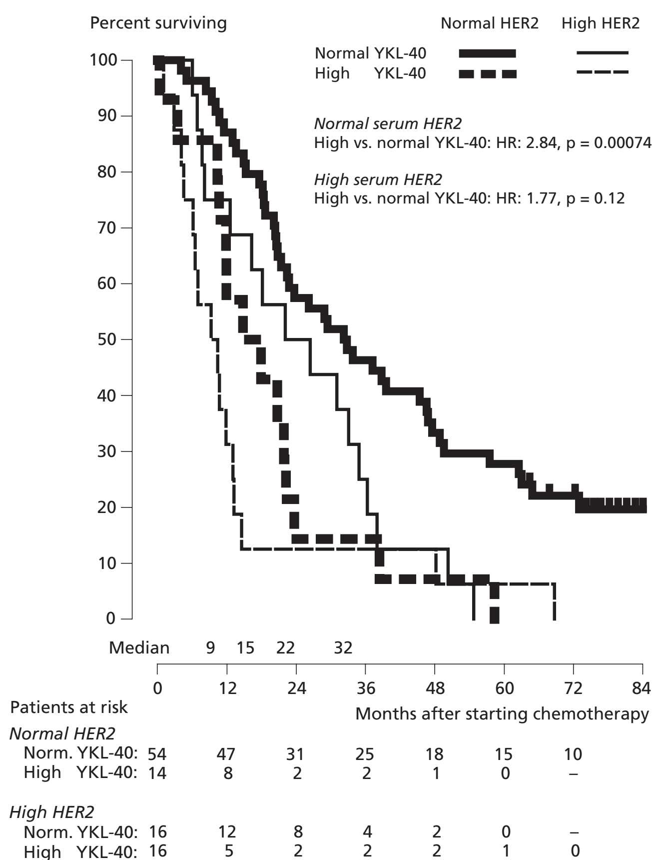

Figure 5. Survial curves in relation to serum concentations of HER2 and YKL-40 in 100 patients with first metastatic manifestation of breast cancer before first line anthracycline-based chemotherapy (Jensen et al. 2003). With a normal serum HER2 level (fat lines), the serum YKL-40 level separated the patients into those with a good prognosis (normal serum YKL-40, straight fat line) and bad prognosis (high serum YKL-40, dotted fat line).

2003). In patients with small cell lung cancer a high serum YKL-40 at time of diagnosis and before chemotherapy was a parameter for death within the following 6 months and was independent of age, sex, disease stage, performance status, and serum LDH (Johansen et al. 2004).

In all six different types of carcinoma tested a high serum YKL-40 level was related to poor prognosis, and serum YKL-40 was independent of other known prognosticators when tested in multivariate Cox analysis. These results suggest that serum YKL-40 may be a useful "prognosticator" identifying a subgroup of cancer patients with a poor prognosis. The function of YKL-40 in cancer diseases is unknown, but these clinical studies indicate that the elevated serum YKL-40 level found in some cancer patients reflects YKL-40 secretion from a subset of tumors with a more aggressive phenotype and a poor prognosis. It is of major importance to evaluate if YKL-40 has a role in promoting growth, invasion and metastasis potential of the cancer cells.

"The marker's degree of expression correlates with therapeutic results (useful for monitoring)"?

One study of curatively operated colorectal cancer patients has evaluated changes in serum YKL-40 levels during the follow-up after surgery (Cintin et al. 2002). It was found that patients with elevated serum YKL-40 six months after the operation had significantly shorter recurrence free interval and overall survival than patients with normal serum YKL-40 at 6 months postoperative. This result was independent of serum CEA levels at 6 months postoperative. Multivariate Cox analysis scoring serum YKL-40 as a time-dependent covariant and including age, Dukes' stage, gender, and tumor localization showed that a high serum YKL-40 postoperatively in curatively operated colorectal cancer patients increased the risk of recurrence within the following 6 months by 6.9 fold and the risk of death by 8.5 fold.

The result of this study indicates that serum concentrations of YKL-40 may be useful for monitoring of cancer patients. However, large longitudinal studies of patients with other types of cancer are needed to evaluate if determination of serum YKL-40 in combination with other prognostic tumor biomarkers can be useful to monitor in cancer patients after primary operation, adjuvant chemo-, antihormonal-, and radio-therapy in order to detect first recurrence early. It is unknown if pretreatment serum YKL-40 levels or early changes during treatment can help to identify patients who respond or do not respond to the given therapy. Longitudinal studies are also needed to evaluate if serum YKL-40 can provide clinical information about disease progression in patients with metastatic cancer before this is detected by routine methods. The present studies show elevated serum YKL-40 particularly in patients with metastases in the liver and lung.

YKL-40 AND LIVER METASTASES

Liver metastases imply a poor prognosis and often develop in patients with breast-, colorectal-, ovarian-, and lung- carcinoma after removal of the primary tumor. High serum YKL-40 levels are often found in patients with liver metastases (Johansen et al. 1995; Cintin et al. 1999, 2002; Dehn et al. 2003; Jensen et al. 2003). The development of liver metastases involves several steps, i.e. exfoliation of cancer cells from the primary site, entry into the portal system, adhesion to the endothelium and subsequent extravasation in the hepatic microvasculature, and multiplication and formation of glandular or acinar structure in the liver parenchyma. Whether or not cancer cells successfully metastasize to the liver depends not only on their cytological properties but also on the hepatic microenvironment involving macrophages and natural killer cells, which constitute the defense system of the liver. Myofibroblasts are usually associated with cancers of epithelial origin and contribute to the growth of metastatic tumors before neovascularization is induced (Schmitt-Graff et al. 1994). Hepatic stellate cells (HSCs) are the only

mesenchymal cells present in the extravascular space of the liver parenchyma. While quiescent in the steady state, they are activated by various stimuli and undergo transformation into myofibroblasts (see also Chapter 4.4). HSCs have been shown to be activated and accumulated around the tumor cells in liver metastases from human colon carcinoma, but the complete interaction between cancer cells and HSC is not well understood. Cancer cells that metastasize to the liver release HSC activating factors and may contribute to the progression of hepatic metastasis. Furthermore growth factors (e.g. PDGF and hepatocyte growth factor) secreted by HSCs augment proliferation and migration of cancer cells *in vitro* (Olaso et al. 1997; Shimizu et al. 2000; Lunevicius et al. 2001). Hepatic myofibroblasts promote the proliferation of hepatocellular carcinoma cell lines, and the latter cells in turn activate and promote proliferation of the former cells (Neaud et al. 1997; Faouzi et al. 1999) demonstrating bi-directional interactions between cancer cells and HSCs. YKL-40 stimulates fibroblasts *in vitro* (Recklies et al. 2002) and one could speculate that YKL-40 secreted by metastatic tumor cells in the liver has an effect on HSCs and myofibroblasts, and that YKL-40 secreted from HSCs activates the cancer cells.

CONCLUSIONS AND FUTURE PERSPECTIVES

In order to propose guidelines on how promising tumor markers progress from the laboratory into the clinic, Hayes and colleagues have introduced the "Tumor Marker Utility Grading System" (TMUGS) (Hayes et al. 1996, 1998). According to this system, serum YKL-40 is on the "Utility scale +" or "Utility scale +/-". YKL-40 is neither organ nor tumor specific, but the present eleven retrospective clinical studies of 1605 patients with different types of cancer indicate that serum concentrations of YKL-40 may be useful as a "prognosticator" and may also have a role in screening and monitoring of cancer patients. Elevated serum concentrations of YKL-40 were found in a subgroup of patients with seven different types of solid carcinoma (including several types of adenocarcinomas, small cell carcinoma, and glioblastoma). The highest serum YKL-40 levels were found in patients with metastatic cancer and with the poorest prognosis and serum YKL-40 provided independent information of survival. The potential values of serum YKL-40 as a biomarker in monitoring and diagnosis of cancer need more studies, and its role as a biomarker in hematological malignancies has to be determined.

According to the "TMUGS", a number of validation requirements are suggested which have to be fulfilled before the marker can be considered to have reached level of evidence I ("LOE I"), whereupon clinical implementation is feasible. Most tumor marker studies are "LOE III", defined as retrospective studies where samples are not originally collected with the intent of testing the value (e.g. prognostic value) of the marker of interest. The intermediate level "LOE II" is constituted by companion studies with prospectively collected specimens as part of a therapeutic trial with pre-established endpoints and evaluation of both the marker and the therapeutic intervention. Finally "LOE I" studies are either 1) highly-powered prospective studies specifically addressing the issue of the utility of the marker or 2) an overview or meta-analysis of studies, each of which have a lower level of evidence.

It is yet unknown if knowledge of the serum YKL-40 level in an individual patient can be reliably used to make clinical decisions that will improve the outcome of the patient. All the present studies of cancer patients regarding serum YKL-40 as a tumor marker in cancer patients are retrospective in design and include a fairly small number of patients and are of a lower level of evidence "LOE III". There are therefore limitations to the conclusions that can be made from the present studies. The prognostic value of serum YKL-40 levels in patients with different types of primary and advanced carcinoma should be confirmed in additional large retrospective studies from other research groups. If a sufficient number of large retrospective studies of high quality confirm the association between serum YKL-40 and poor prognosis a summarising meta-analysis can bring the generated data to "LOE I". According to the "TMUGS" guidelines, the next step would be to launch an appropriate prospective study where the benefit of using serum YKL-40 levels in the clinical decision-making process is assessed. Endpoints should include overall survival, disease-free survival, quality of life and cost-effectiveness. The study could be designed either as a single, highly-powered, prospective, controlled study with the primary objective of testing serum YKL-40 level as a "prognosticator" or a similar prospective study where the primary goal could be the testing of a therapeutic hypothesis and secondly testing serum YKL-40 as a biomarker.

A major issue to explore is the question: "Can YKL-40 or its receptor(s) be potential targets for cancer therapy?". Unfortunately, the biological function of YKL-40 in cancer development and metastases is unknown and the elucidation of a possible function of YKL-40 in cancer diseases is an important objective of future studies. It has been shown that YKL-40 exhibits growth factor activity for cell types involved in tissue remodeling processes, and it has been suggested that YKL-40 has a role in cancer cell growth and survival, the inflammatory process around the tumor, angiogenesis, and remodeling of the ECM surrounding the cancer cells as pointed out previously. Based on the present clinical studies of serum YKL-40 levels in cancer patients one could hypothesize that YKL-40 will prove to have a role in the ability of cancer cells to proliferate, survive, invade and metastasize and/or a regulating role in cancer cell-matrix interactions and in the production of the altered extracellular matrix surrounding the cancer cells. Provided future studies show that YKL-40 has such roles, YKL-40 could be an attractive target in the design of anticancer therapy. Any approach that would inhibit the function of YKL-40 (e.g. inhibition of YKL-40 gene expression, protein synthesis and secretion, neutralization of YKL-40 activity, blocking YKL-40 conversion from a latent to an active form, interruption of YKL-40 affinity or reaction with its receptor) may limit cancer growth and metastases and improve the survival of cancer patients with YKL-40 expressing tumor cells. Potential inhibitors of YKL-40 activity include methods to inhibit YKL-40 production (e.g. siRNA), human (or humanized) monoclonal antibodies specific for YKL-40 or its receptor(s), YKL-40 receptor antagonists, or substrate molecules that competitively bind to YKL-40. Such potential inhibitors of YKL-40 could be expected to have therapeutic efficacy in cancer patients with tumors that produce YKL-40. It is therefore of major importance to explore if YKL-40 could become a target for the development of new cancer therapeutics.

6. GENERAL CONCLUSIONS

The purpose of this thesis was to determine if serum YKL-40 is a clinically useful biomarker of disease activity and prognosis in human disease. The results of these studies indicate that serum YKL-40 is a biomarker of pathogenic processes related to inflammation, extracellular tissue remodeling, fibrosis and solid carcinomas. Although these results must be confirmed in large, prospective clinical studies of patients with each of these diseases, several general conclusions can be made:

YKL-40 is expressed and secreted by inflammatory cells (neutrophils, a subgroup of monocytes/macrophages, giant cells), chondrocytes, fibroblast-like synovial cells, vascular smooth muscle cells, endothelial cells, hepatic stellate cells and by malignant cells from many different solid carcinomas. Increased expression of YKL-40 mRNA and protein in human tissues is found in pathological conditions with acute or chronic inflammation, increased remodeling of the ECM, development of fibrosis and cancer as illustrated by the increased local synthesis of YKL-40 in affected tissue from patients with meningitis, rheumatoid arthritis, osteoarthritis, giant cell arteritis, sarcoidosis, scleroderma, liver fibrosis and solid carcinoma.

YKL-40 concentrations can be measured in conditioned media of human cell cultures and in human serum, EDTA plasma, and synovial fluid by RIA or ELISAs. The serum concentration of YKL-40 is

stable at a low level in healthy children, young and middle age adults, but increases in healthy elderly, probably due to increased subclinical inflammation or to an undiscovered disease. Elevated serum YKL-40 levels (i.e. higher than the 95th level in age-matched healthy controls) are found in patients with different pathological conditions characterized by either acute or chronic inflammation, increased remodeling of the ECM, development of fibrosis and cancer. This is illustrated in studies of patients with the following diseases: acute bacterial infections, rheumatoid arthritis, osteoarthritis, giant cell arteritis, sarcoidosis, scleroderma, inflammatory bowel disease, liver fibrosis, and seven different types of solid carcinoma. These studies found that serum YKL-40 reflects disease activity in the patients.

The prognostic value of serum YKL-40 was studied in patients with *Streptococcus pneumoniae* bacteremia, rheumatoid arthritis, alcoholic liver disease and six different types of solid carcinomas. All studies found that an elevated serum YKL-40 level is a prognostic biomarker of a poor prognosis. All but one of the studies further found that serum YKL-40 provided independent prognostic information when compared with other known prognostic biomarkers. Serum concentrations of YKL-40 appear to reflect other aspects of inflammation than serum CRP in patients with acute bacterial infections, rheumatoid arthritis, giant cell arteritis, and inflammatory bowel disease. Large prospective studies of serum YKL-40 and CRP levels in patients with rheumatoid arthritis should be performed to assess if the combination of these 2 parameters is more useful to determine ongoing disease activity and to predict joint destruction than if only serum CRP is determined. In patients with liver disease the serum YKL-40 level was useful to discriminate between the absence and presence of fibrosis. Large prospective studies of patients with different liver diseases are needed to investigate if determination of serum YKL-40 in combination with other circulating biomarkers of connective tissue metabolism (e.g. serum hyaluronan and PIIINP) can be used in clinical practice for detection and monitoring of liver fibrosis. In patients with solid carcinomas the serum concentration of YKL-40 provided information of disease extension and aggressiveness and serum YKL-40 was not closely related to other serological tumor markers. High serum YKL-40 levels in cancer patients with six different types of solid carcinoma was found to be a "prognosticator" of short time to disease progression and short survival and one study also suggests that serum YKL-40 has a potential value in monitoring of cancer patients. These results have to be confirmed in large prospective studies of cancer patients and it needs to be determined if YKL-40 is elevated in serum of patients with hematological malignancies.

The study of YKL-40 has just started and several biological questions regarding this protein remain to be answered. The complete biological function of YKL-40 is unclear, and it is not yet known if YKL-40 has a receptor. The present clinical studies suggest that YKL-40 has a role in pathological growth, metastatic potential, in inflammation and tissue remodelling processes and in pathological conditions leading to fibrosis. The mechanisms by which stimuli lead to increased expression and synthesis of YKL-40 are unknown, however, and deserve intensive studies. YKL-40 knock-out mice or transgenic mice are not described in the literature and will hopefully be made in the near future. *In vitro* studies have found that YKL-40 promotes the growth of fibroblasts, endothelial cells, fibroblast-like synovial cells and chondrocytes, and works in a synergistic fashion with IGF-1. It has been suggested, but not proven, that YKL-40 has an anti-apoptotic function. In rheumatoid arthritis YKL-40 seems to be an autoantigen with a possible role in the pathogenesis of rheumatoid arthritis. It remains to be determined whether YKL-40 is related to the autoimmune response underlying some of the other autoimmune diseases. YKL-40 is expressed by cancer cells from solid carcinomas and by tumor-associated macrophages but its function in cancer development, growth and metastasis are unknown.

It has been challenging and rewarding to investigate the potential of serum YKL-40 as a biomarker in human disease. It is also exciting to see the steady increase in the number of other investigators who have studied the biomarker potential of YKL-40 and have consistently found evidence for clinical utility in serum YKL-40 measurement. Since there are no available biomarkers that provide the same clinical information in human disease as YKL-40, there is reason to be optimistic that it will have a place in the routine clinical management of a number of human diseases. The protein is more than a biomarker, however. It is a protein secreted by cells involved in a variety of human diseases, and its function, when known, should provide a basis for better understanding of these disease processes. It is also possible that the protein itself may prove to be a therapeutic target for human disease.

Abbreviations

38-kDa heparin-binding glycoprotein (Gp38k)
40 kDa mammary gland protein (MGP-40)
acidic mammalian chitinase (AMCase)
alpha-fetoprotein (AFP)
alanine (A)
American college of rheumatology (ACR)
aminoterminal propeptide of type I procollagen (PINP)
aminoterminal propeptide of type III procollagen (PIIINP)
angiotensin-converting enzyme (ACE)
ankylosing spondylitis (AS)
arginine (R)
asparagine (Asn)
aspartic acid (D)
basic fibroblast growth factor (bFGF)
bronchioalveolar lavage (BAL) fluid
bone morphogenic proteins (BMP)
breast regressing protein 39 Kd (brp-39)
C-reactive protein (CRP)
carbon monoxide diffusion capacity corrected
for alveolar volume (D_LCO/VA)
carcinoembryonic antigen (CEA)
cartilage oligomeric matrix protein (COMP)
chitinase-3-like-1 (CHI3L1)
coefficient of variation (CV)
complementary deoxyribonucleic acid (cDNA)
Crohn's disease (CD)
cysteine (C)
dalton (Da)
disease activity score (DAS)
disease modifying antirheumatic drugs (DMARD's)
disability index of the health assessment questionnaire (HAQ)
enzyme-linked immunoassay (ELISA)
eosinophil chemotactic cytokine (ECF-L)
epidermal growth factor (EGF)
erythrocyte sedimentation rate (ESR)
European league against rheumatism (EULAR)
extracellular matrix (ECM)
giant cell arteritis (GCA)
glutamic acid (E)
glutamine (Q)
glycine (G)
granulocyte-macrophage colony-stimulating factor (GM-CSF)
hepatic stellate cell (HSC)
hepatitis C virus (HCV)
histidine (H)
horseradish peroxidase (HRP)
hour (h)
human cartilage glycoprotein-39 (HC gp39)
human chorionic gonadotropin (hCG)
human epidermal growth factor receptor 2 (HER-2)

imaginal disc growth factors (IDGFs)
inducible silicotic bronchoalveolar lavage protein-p^{58} (iSBLP^{58})
inflammatory bowel disease (IBD)
insulin-like growth factor-1 (IGF-1)
interferon-γ (IFNγ)
interleukin-1 (IL-1)
interleukin-6 (IL-6)
interleukin-8 (IL-8)
lactate dehydrogenase (LDH)
level of evidence (LOE)
leucine (L)
lysine (K)
magnetic resonance imaging (MRI)
major histocompatibility complex (MHC)
messenger ribonucleic acid (mRNA)
metalloproteinase (MMP)
methotrexate (MTX)
mitogen-activated protein (MAP)
N-acetylglucosamine (GlcNAc) residues
osteoarthritis (OA)
percentage (%)
peripheral blood mononuclear cell (PBMC)
phorbol myristate acetate (PMA)
platelet-derived growth factor (PDGF)
polymyalgia rheumatica (PMR)
positive (+)
prostate specific antigen (PSA)
radioimmunoassay (RIA)
receptor activator of nuclear factor (NF)-κB ligand (RANKL)
reverse transcriptase polymerase chain reaction (RT-PCR)
rheumatoid arthritis (RA)
rheumatoid factor (RF)
ritchie articular index (RAI)
serial analysis of gene expression (SAGE)
systemic sclerosis (SSc)
tissue inhibitor of metalloproteinase (TIMP)
transforming growth factor beta (TGFβ)
tumor marker utility grading system (TMUGS)
tumor necrosis factor alfa (TNFα)
triose-phosphate isomerase (TIM)
tyrosine (Y)
ulcerative colitis (UC)
ultrasound (UL)
vascular endothelial growth factor (VEGF)
visual analogue scale (VAS)

REFERENCES

Abe M, Takahashi M, Naitou K, Ohmura K, Nagano A. Investigation of generalized osteoarthritis by combining X-ray grading of the knee, spine and hand using biochemical markers for arthritis in patients with knee osteoarthritis. Clinical Rheum 2003;22:425-31.

Achen MG, Stacker SA. The vascular endothelial growth factor family; proteins which guide the development of the vasculture. Int J Exp Path 1998;79:255-65.

Achkar AA, Lie JT, Hunder GG, O'Fallon WM, Gabriel SE. How does previous corticosteroid treatment affect the biopsy findings in giant cell (temporal) arteritis? Ann Intern Med 1994;120:987-92.

American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines. Guidelines for the management of rheumatoid arthritis 2002 updata. Arthritis Rheum 2002;46:328-46.

Aigner T, Bertling W, Stoss H, Weseloh G, von der Mark K. Independent expression of fibril-forming collagens I, II, and III in chondrocytes of human osteoarthritic cartilage. J Clin Invest 1993;91:829-37.

Aigner T, Gluckert K, von der Mark K. Activation of fibrillar collagen syntheis and phenotypic modulation of chondrocytes in early human osteoarthritic cartilage lesions. Osteoarthritis Cartilage 1997;5:183-9.

Aigner T, Zhu Y, Chansky HH, Matsen FA III, Maloney WJ, Sandell LJ. Reexpression of type IIA procollagen by adult articular chondorcytes in osteoarthritic cartilage. Arthritis Rheum 1999;42:1443-50.

Allen RKA, Chai SY, Dunbar MS, Mendelsohn FAO. In vitro autoradiographic localisation of angiotensin-converting enzyme in sarcoid lymph nodes. Chest 1986;90:315-20.

Araujo ACG, Souto-Padron T, de Souza W. Cytochemical localization of carbohydrate residues in microfilariae of Wuchereria bancrofti and Brugia malayi. J Histochem Cytochem 1993;41:571-8.

Allen RKA. A review of angiotensin-converting enzyme in health and disease. Sarcoidosis 1991;8:95-100.

Arend WP, Dayer J-M. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor α in rheumatoid arthritis. Arthritis Rheum 1995;38:151-60.

Arias EB, Verhage HG, Jaffe RC. Complementary deoxyribonucleic acid cloning and molecular characterization of an estrogen-dependent human oviductal glycoprotein. Biol Reproduc 1994;51:685-94.

Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr., Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.

Aronson NN, Blanchard CJ, Madura JD. Homology modeling of glycosyl hydrolase family 18 enzymes and proteins. J Chem Inf Comput Sci 1997;37:999-1005.

Atkinson AJ, Jr. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89-95.

Baeten D, Boots AMH, Steenbakkers PGA, Elewaut D, Bos E, Verheijden GFM, Verbruggen G, Miltenburg AMM, Rijnders AWM, Veys EM, de Keyser F. Human cartilage gp-39+,CD16+ monocytes in peripheral blood and synovium. Correlation with joint destruction in rheumatoid arthritis. Arthritis Rheum 2000;43:1233-43.

Baeten D, Steenbakkers PGA, Rijnders AMW, Boots AM, Veys EM, de Keyser F. Detection of major histocompatibility complex/human cartilage gp-39 complexes in rheumatoid arthritis synovitis as a specific and independent histologic marker. Arthritis Rheum 2004;50:444-51.

Bakkenist CJ, Kastan MB. Initiating cellular stress responses. Cell 2004;118: 9-17.

Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539-45.

Ballara S, Taylor PC, Reusch P, Marmé D, Feldmann M, Maini RN, Paleolog EM. Raised serum vascular endothelial growth factor levels are associated with destructive change in inflammatory arthritis. Arthritis Rheum 2001;44:2055-64.

Baptista A, Bianchi L, De Groote J, Desmet VJ, Ishak KG, Korb G, MacSween RN, Popper H, Poulsen H, Scheuer PJ. The diagnostic significance of periportal hepatic necrosis and inflammation. Histopathology 1988;12:569-79.

Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin Science 1998;94:557-72.

Basset P, Bellocq JP, Wolf C, Stoll I, Hutin P, Limacher JM, Podhajcer OL, Chenard MP, Rio MC, Chambon P. A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature 1990;348: 699-704.

Bast RC Jr, Xu FJ, Yu YH, Barnhill S, Zhang Z, Mills GB. CA125: The past and the future. Int J Biol Markers 1998; 13:179-87.

Bast RC, Jr. Status of tumor markers in ovarian cancer screening. J Clin Oncol 2003;21(Suppl 10):200s-5s.

Baumann H, Gauldie J. The acute phase response. Immunol Today 1994;15: 74-80.

Bedossa P, Paradis V. Liver extracellular matrix in health and disease. J Pathol 2003;200:504-15.

Bentsen KD. Type III procollagen peptide: studies on the circulating peptide as a marker of fibrogenesis with special reference to the liver. Thesis. Lægeforeningensforlag, København 1992.

Benya PD, Padilla SR, Nimni ME. Independent regulation of collagen types of chondrocytes during the loss of differentiated function in culture. Cell 1978;15:1313-21.

Benya PD, Shaffer JD. De-differentiated chondrocytes re-express the differentiated collagen phenotype when cultured in agarose gels. Cell 1982;30: 373-84.

Benyon RC, Arthur MJP. Extracellular matrix degradation and the role of hepatic stellate cells. Semin Liver Dis 2001; 21:373-84.

Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nature Reviews 2003;3:401-10.

Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol 2002;196:254-65.

Bissell MJ, Radisky D. Putting tumours in context. Nat Rev Cancer 2001;1: 46-54.

Bläss S, Engel JM, Burmester GR. The immunologic homunculus in rheumatoid arthritis. Arthritis Rheum 1999;42: 2499-506.

Bodamyali T, Stevens CR, Billingham ME, Ohta S, Blake DR. Influence of hypoxia in inflammatory synovitis. Ann Rheum Dis 1998;57:703-10.

Boers M, Kostense PJ, Verhoeven AC, van der Linden S for the COBRA Trial Group. Inflammation and damage in an individual joint predict further

damage in that joint in patients with early rheumatoid arthritis. Arthritis Rheum 2001;44:2242-6.
Bokma E, Rozeboom HJ, Sibbald M, Dijkstra BW, Beintema JJ. Expression and characterization of active site mutants of hevamine, a chitinase from the rubber tree Hevea brasiliensis. Eur J Biochem 2002;269:893-901.
Bonaventure J, Kadhom N, Cohen-Solal L, Ng KH, Bourguignon J, Lasselin C. Reexpression of cartilage-specific genes by dedifferentiated human articular chondrocytes cultured in alginate beads. Exp Cell Res 1994;212: 97-104.
Boot RG, Renkema GH, Strijland A, van Zonneveld AJ, Aerts JMFG. Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. J Biol Chem 1995;270:26252-6.
Boot RG, Renkema GH, Verhoek M, Strijland A, Bliek J, de Meulemeester TMAMO, Mannen MMAM, Aerts JMFG. The human chitotriosidase gene. Nature of inherited enzyme deficiency. J Biol Chem 1998;273:25680-5.
Boot RG, van Achterberg AE, van Aken BE, Renkema GH, Jacobs MJHM, Aerts JMFG, de Vries CJM. Strong induction of members of the chitinase family of proteins in atherosclerosis. Chitotriosidase and human cartilage gp-39 expressed in lesion macrophages. Arterioscler Thromb Vasc Biol 1999;19:687-94.
Boot RG, Blommaart EFC, Swart E, Ghauharali-van der Vlugt K, Bijl N, Moe C, Place A, Aerts JMFG. Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J Biol Chem 2001;276:6770-8.
Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. Blood 1997;89:3503-21.
Bosl GJ, Motzer RJ. Testicular germ-cell cancer. N Engl J Med 1997;337:242-53.
Boudreau N, Bissell MJ. Extracellular matrix signaling: integration of form and function in normal and malignant cells. Cur Opin Cell Biol 1998;10: 640-6.
Boussac M, Garin J. Calcium-dependent secretion in human neutrophils: a proteomic approach. Electrophoresis 2000; 21:665-72.
Brack A, Rittner HL, Younge BR, Kaltschmidt C, Weyand CM, Goronzy JJ. Glucocorticoid-mediated repression of cytokine gene transcription in human arteritis-SCID chimeras. J Clin Invest 1997;99:2842-50.
Brasso K, Christensen IJ, Johansen JS, Teisner B, Garnero P, Price PA, Iversen P. Prognostic value of PINP, bone alkaline phosphatase, CTX-I, and YKL-40 in patients with metastatic prostate carcinoma. Prostate 2006;66:503-13.
Braun J, Bollow M, Sieper J. Radiologic diagnosis and pathology of the spondyloarthropathies. Rheum Dis Clin North AM 1998;24:697-735.
Braun J, Khan MA, Sieper J. Enthesitis and ankylosis in spondyloarthropathy: what is the target of the immune response? Ann Rheum Dis 2000;59:985-94.
Breedveld FC, Dayer J-M. Leflunomide: mode of action in the treatment of rheumatoid arthritis. Ann Rheum Dis 2000; 59:841-9.
Brennan FM, Maini RN, Feldmann M. TNFα – a pivotal role in rheumatoid arthritis? Br J Rheumatol 1992;31:293-8.
Brennan P, Harrison B, Barrett E, Chakravarty K, Scott D, Silman A, Symmons D. A simple algorithm to predict the development of radiological erosions in patients with early rheumatoid arthritis: prospective cohort study. BMJ 1996; 313:471-6.
Bruunsgaard H, Pedersen M, Pedersen BK. Aging and proinflammatory cytokines. Curr Opin Hematol 2001;8:131-6.
Buckwalter JA, Mankin HJ. Articular cartilage. Part II: Degeneration and osteoarthrosis, repair, regeneration, and transplantation. J Bone Joint Surg Am 1997;79:612-32.
Buhi WC. Characterization and biological roles of oviduct-specific, oestrogen-dependent glycoprotein. Reproduction 2002;123:355-62.
Bukhari M, Harrison B, Lunt M, Scott DGI, Symmons DPM, Silman AJ. Time to first occurrence of erosions in inflammatory polyarthritis: results from a prospective community-based study. Arthritis Rheum 2001;44: 1248-53.
Bundgaard H, Kjeldsen K, Krabbe KS, van Hall G, Simonsen L, Qvist J, Hansen CM, Møller K, Fonsmark L, Madsen PL, Pedersen BK. Endotoxemia stimulates skeletal muscle Na^+-K^+-ATPase and raises blood lactate under aerobic conditions in humans. Am J Physiol Heart Circ Physiol 2003; 284:H1028-34.
Buttgereit F, Straub RH, Wehling M, Burmester G-R. Glucocorticoids in the treatment of rheumatoid diseases. An update on the mechanism of action. Arthritis Rheum 2004; 50:3408-17.
Canto EI, Shariat SF, Slawin KM. Molecular diagnosis of prostate cancer. Curr Urol Rep 2004;5:203-11.
Cao WW, Kao PN, Aoki Y, Xu JC, Shorthouse RA, Morris RE. A novel mechanism of action of the immunoregulatory drug, leflunomide: augmentation of the immunosuppressive cytokine, TGF-beta 1, and supression of the immunostimulatory cytokine, IL-2. Transplant Proc 1996;28:3079-80.
Carney WP. The emerging role of monitoring serum HER-2/neu oncoprotein levels in women with metastatic breast cancer. Lab Med 2003;34:58-64.
Castell JV, Gomez-Lechon MJ, David M, Fabra R, Trullenque R, Heinrich PC. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. Hepatology 1990;12:1179-86.
Catalona WJ, Richie JP, Ahmann FR, Hudson MA, Scardino PT, Flanigan RC, DeKernion JB, Ratliff TL, Kavoussi LR, Dalkin BL, Waters WB, MacFarlane MT, Southwick PC. Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6630 men. J Urol 1994;151:1283-90.
Chang N-CA, Hung S-I, Hwa K-Y, Kato I, Chen J-E, Liu C-H, Chang AC. A macrophage protein, Ym1, transiently expressed during inflammation is a novel mammalian lectin. J Biol Chem 2001;276:17497-506.
Charles PJ, Maini RN. Serum YKL-40, a chondrocyte derived protein, is reduced by infliximab (anti TNF) therapy in patients with rheumatoid arthritis. Arthritis Rheum 1999;42 (Suppl): abstract 994.
Chatman WW, Swain R, Frohsin H Jr, Heck LW, Miller EJ, Blackburn WD Jr. Degradation of human articular cartilage by neutrophils in synovial fluid. Arthritis Rheum 1993;36:51-8.
Chikanza LC, Panayi GS. The effects of hydrocortisone on in vitro lymphocyte proliferation and interleukin-2 and -4 production in corticosteroid sensitive and resistant subjects. Eur J Clin Invest 1993;23:845-50.
Choy EHS, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 2001;344: 907-16.
Choy EHS, Isenberg DA, Garrood T, Farrow S, Ioannou Y, Bird H, Cheung N, Williams B, Hazleman B, Price R, Yoshizaki K, Nishimoto N, Kishimoto T, Panayi GS. Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis. Arthritis Rheum 2002;46:3143-50.
Chung C, Tallerico T, Seeman P. Schizophrenia hippocampus has elevated expression of chondrex glycoprotein gene. Synapse 2003;50:29-34.
Cintin C, Johansen JS, Christensen IJ, Price PA, Sørensen S, Nielsen HJ. Serum YKL-40 and colorectal cancer. Br J Cancer 1999;79:1494-9.
Cintin C, Johansen JS, Skov F, Price PA, Nielsen HJ. Accumulation of the neutrophil-derived protein YKL-40 during storage of various blood components. Inflammation Res 2001;50:107-11.
Cintin C, Johansen JS, Christensen IJ, Price PA, Sørensen S, Nielsen HJ. High serum YKL-40 level after surgery for colorectal carcinoma is related to short survival. Cancer 2002;95:267-74.
Clasper S, Vekemans S, Fiore M, Plebanski M, Wordsworth P, David G, Jackson DG. Inducible expression of the cell surface heparan sulfate proteoglycan-2 (fibroglycan) on human activated macrophages can regulate fibroblast growth factor action. J Biol Chem 1999;274:24113-23.
Collinge DB, Kragh KM, Mikkelsen JD, Nielsen KK, Rasmussen U, Vad K. Plant chitinases. Plant J 1993;3:31-40.
Combe B, Dougados M, Goupille P, Cantagrel A, Eliaou JF, Sibilia J, Meyer O, Sany J, Daures J-P, Dubois A. Prognostic factors for radiographic damage in early rheumatoid arthritis: a multiparameter prospective study. Arthritis Rheum 2001;44:1736-43.
Compton C, Fenoglio-Preiser CM, Pettigrew N, Fielding LP. American joint committee on cancer prognostic factors consensus conference. Colorectal working group. Cancer 2000;88:1739-57.
Connor JR, Dodds RA, Emery JG, Kirkpatrick RB, Rosenberg M, Gowen M. Human cartilage glycoprotein 39 (HC gp-39) mRNA expression in adult and fetal chondrocytes, osteoblasts and osteocytes by in-situ hybridization. Osteoarthritis Cartilage 2000;8:87-95.
Conrozier T, Saxne T, Shan Sei Fan C, Mathieu P, Tron A-M, Heinegard D et al. Serum concentrations of cartilage oligomeric matrix protein and bone sialoprotien in hip osteoarthritis: a one year prospective study. Ann Rheum Dis 1998;9:527-32.
Conrozier Th, Carlier M-C, Mathieu P, Colson F, Debard AL, Richard S, Favret H, Bienvenu J, Vignon E. Serum levels of YKL-40 and C reactive protein in patients with hip osteoarthritis and healthy subjects: a cross sectional study. Ann Rheum Dis 2000;59:828-31.
Cope AP, Patel SD, Hall F, Congia M, Hubers HAJM, Verheijden GF, Boots AMH, Menon R, Trucco M, Rijnders AWM, Sønderstrup G. T cell responses to a human cartilage autoantigen in the context of rheumatoid arthritis-associated and nonassociated HLA-DR4 alleles. Arthritis Rheum 1999;42:1497-507.
Coulson AFW. A proposed structure for “family 18” chitinases. A possible function for narbonin. FEBS Lett 1994; 354:41-4.
Cronstein BN. Molecular therapeutics: methotrexate and its mechanism of action. Arthritis Rheum 1996;39:1951-60.
D'Amore M, Germinario G, D'Amore S, Scagliusi P. Marcatori biochimici di turnover osseo e YKL-40 nella spondilite anchilosante. Minerva Med 2000;91:59-68.
Dasgupta B, Corkill M, Kirkahm B, Gibson T, Panayi G. Serial estimation of interleukin 6 as a measure of systemic disease in rheumatoid arthritis. J Rheumatol 1992;19:22-5.
Dasuri K, Antonovici M, Chen K, Wong K, Standing K, Ens W, El-Gabalawy H, Wilkins JA. The synovial proteome: analysis of fibroblast-like synoviocytes. Arthritis Res Ther 2004;6:R161-8.
Debono M, Gordee RS. Antibiotics that inhibit fungal cell wall development. Annu Rev Microbiol 1994;48:471-97.

De Ceuninck F, Pastoureau P, Bouet F, Bonnet J, Vanhoutte PM. Purification of guinea pig YKL-40 and modulation of its secretion by cultured articular chondrocytes. J Cell Biochem 1998;69:414-24.

De Ceuninck F, Gaufillier S, Bonnaud A, Sabatini M, Lesur C, Pastoureau P. YKL-40 (Cartilage gp-39) induces proliferative events in cultured chondrocytes and synoviocytes and increases glycosaminoglycan synthesis in chondrocytes. Biochem Biophys Res Commun 2001a;285:926-31.

De Ceuninck F, Pastoureau P, Agnellet S, Bonnet J, Vanhoutte PM. Development of an enzyme-linked immunoassay for the quantification of YKL-40 (cartilage gp-39) in guinea pig serum using hen egg yolk antibodies. J Immunol Methods 2001b;252:153-61.

Dehn H, Høgdall EV, Johansen JS, Jørgensen M, Price PA, Engelholm SA, Høgdall CK. Plasma YKL-40, as a prognostic tumor marker in reccurent ovarian cancer. Acta Obstet Gynecol Scand 2003;82:287-93.

den Broeder AA, Joosten LAB, Saxne T, Heinegård D, Fenner H, Miltenburg AMM, Frasa WLH, van Tits LJ, Buurman WA, van Riel PLCM, van de Putte LBA, Barrera P. Long term anti-tumour necrosis factor α monotherapy in rheumatoid arthritis: effect on radiological course and prognostic value of markers of cartilage turnover and endothelial activation. Ann Rheum Dis 2002;61:311-8.

Desgeorges A, Gabay C, Silacci P, Novick D, Roux-Lombard P, Grau G, Dayer J-M, Vischer T, Guerne P-A. Concentrations and origins of soluble interleukin 6 receptor-α in serum and synovial fluid. J Rheumatol 1997;24: 1510-6.

Dolhain RJEM, Tak PP, Dijkmans BAC, De Kuiper P, Breedveld FC, Miltenburg AMM. Methotrexate treatment reduces inflammatory cell numbers, expression of monokines and of adhesion molecules in synovial tissue of patients with rheumatoid arthritis. Br J Rheumatol 1998;37:502-8.

Downward J. PI 3-kinase, Akt and cell survival. Semin Cell Dev Biol 2004;15:177-82.

DuClos TW. Function of C-reactive protein. Ann Med 2000; 32:274-8.

Duffy MJ, van Dalen A, Haglund C, Hansson L, Klapdor R, Lamerz R, Nilsson O, Sturgeon C, Topolcan O. Clinical utility of biochemical markers in colorectal cancer: European group on tumour markers (EGTM) guidelines. Eur J Cancer 2003;39:718-27.

Dupont J, Tanwar MK, Thaler HT, Fleisher M, Kauff N, Hensley ML, Sabbatini P, Anderson S, Aghajanian C, Holland EC, Spriggs DR. Early detection and prognosis of ovarian cancer using serum YKL-40. J Clin Oncol 2004; 22:3330-9.

Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315:1650-9.

Eberhardt K, Fex E. Clinical course and remission rate in patients with early rheumatoid arthritis: relationship to outcome after 5 years. Br J Rheumatol 1998;37:1324-9.

Emery P, Luqmani R. The validity of surrogate markers in rheumatic disease. Br J Rheumatol 1993;32(Suppl 3):3-8.

Faouzi S, Lepreux S, Bedin C, Dubuisson L, Balabaud C, Bioulac-Sage P, Desmouliere A, Rosenbaum J. Activation of cultured rat hepatic stellate cells by tumoral hepatocytes. Lab Invest 1999;79:485-93.

Farahat MN, Yanni G, Poston R, Panayi GS. Cytokine expression in synovial membranes of patients with rheumatoid arthritis and osteoarthritis. Ann Rheum Dis 1993;52:870-5.

Faurschou M, Borregaard N. Neutrophil granules and secretory vesicles in inflammation. Microbes Infections 2003;5: 1317-27.

Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, Katz LM, Lightfoot R Jr, Paulus H, Strand V, Tugwell P, Weinblatt M, Williams HJ, Wolfe F, Kieszak S. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995;38:727-35.

Ferrara N. VEGF and the quest for tumour angiogenesis factors. Nature Reviews 2002;2:795-803.

Ferrara N, Gerber H-P, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669-76.

Fex E, Jonsson K, Johnson U, Eberhardt K. Development of radiographic damage during the first 5-6 years of rheumatoid arthritis. A prospective follow-up study of a Swedish cohort. Br J Rheumatol 1996;35:1106-15.

Fiore CE, Tamborino C. YKL-40 and graft rejection. Am J Med 2000;108: 688-9.

Firestein GS, Paine MM, Littman BH. Gene expression (collagenase, tissue inhibitor of metalloproteinases, complement, and HLA-DR) in rheumatoid arthritis and osteoarthritis synovium: quantitative analysis and effect of intraarticular corticosteroids. Arthritis Rheum 1991;34: 1094-105.

Firestein GS, Paine MM, Boyle DL. Mechanisms of methotrexate action in rheumatoid arthritis: selective decrease in synovial collagenase gene expression. Arthritis Rheum 1994;37:193-200.

Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423: 356-61.

Flach J, Pilet P-E, Jolles P. What's new in chitinase research? Experientia 1992;48:701-16.

Fingerle G, Pforte A, Passlick B, Blumenstein M, Ströbel M, Ziegler-Heitbrock HWL. The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients. Blood 1993;82:3170-6.

Fiore CE, Pennisi P, Tamborina C. YKL-40 and graft rejection. Letter. Am J Med 2000;108:688-9.

Forslind K, Eberhardt K, Jonsson A, Saxne T. Increased serum concentrations of cartilage oligomeric matrix protein. A prognostic marker in early rheumatoid arthritis. Br J Rheumatol 1992;31:593-8.

Franceschi RT, Romano PR, Park K-Y. Regulation of type I collagen synthesis by 1,25-dihydroxyvitamin D3 in human osteosarcoma cells. J Biol Chem 1988;263:18938-45.

Frankenberger M, Sternsdorf T, Pechumer H, Pforte A, Ziegler-Heitbrock HWL. Differential cytokine expression in human blood monocyte subpopulations: a polymerase chain reaction analysis. Blood 1996;87:373-7.

Freed LE, Vunjak-Novakovic G, Langer R. Cultivation of cell-polymer cartilage implants in bioreactors. J Cell Biochem 1993;51:257-64.

Freemont AJ, Hampson V, Tilman R, Goupille P, Taiwo Y, Hoyland JA. Gene expression of matrix metalloproteinases 1,3, and 9 by chondrocytes in osteoarthritis human knee articular cartilage is zone and grade specific. Ann Rheum Dis 1997;56:542-9.

Friedman SL. The cellular basis of hepatic fibrosis: mechanisms and treatment strategies. N Engl J Med 1993;328: 1828-35.

Friedman SL. Stellate cell activation in alcoholic fibrosis – an overview. Alcohol Clin Exp Res 1999;23:904-10.

Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000;275:2247-50.

Fusetti F, von Moeller H, Houston D, Rozeboom HJ, Dijkstra BW, Boot RG, Aerts JMFG, van Aalten DMF. Structure of human chitotriosidase. Implications for specific inhibitor design and function of mammalian chitinase-like lectins. J Biol Chem 2002;277:25537-44.

Fusetti F, Pijning T, Kalk KH, Bos E, Dijkstra BW. Crystal structure and carbohydrate-binding properties of the human cartilage glycoprotein-39. J Biol Chem 2003;278: 37753-60.

Gabay S, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448-54.

Gabriel SE, Crowson CS, O'Fallon WM. Mortality in rheumatoid arthritis: have we made an impact in 4 decades? J Rheumatol 1999;26:2529-33.

Ganz T, Lehrer RI. Antimicrobial peptides of leukocytes. Curr Opin Hematol 1997;4:53-8.

Garnero P, Jolvenne P, Delmas P, Miossec P. Serum YKL-40 and joint destruction in patients with rheumatoid arthritis. EULAR congres 1999.

Garnero P, Rousseau J-C, Delmas PD. Molecular basis and clinical use of biochemical markers of bone, cartilage, and synovium in joint diseases. Arthritis Rheum 2000;43:953-68.

Garnero P, Piperno M, Gineyts E, Christgau S, Delmas PD, Vignon E. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. Ann Rheum Dis 2001;60:619-26.

Garnero P, Ayral X, Rousseau J-C, Christgau S, Sandell LJ, Dougados M, Delmas PD. Uncoupling of type II collagen synthesis and degradation predicts progression of joint damage in patiens with knee osteoarthritis. Arthritis Rheum 2002a;2613-24.

Garnero P, Gineyts E, Christgau S, Finck B, delmas PD. Association of baseline levels of urinary glucosyl-galactosylpyridinoline and type II collagen C-telopeptide with progression of joint destruction in patients with early rheumatoid arthritis. Arthritis Rheum 2002b;46:21-30.

Garnero P, Landewe R, Boers M, Verhoeven A, vand der Linden S, Christgau S, van der Heijde D, Boonen A, Geusens P. Association of baseline levels of markers of bone and cartilage degradation with long-term progression of joint damage in patients with early rheumatoid arthritis. The Cobra Study. Arthritis Rheum 2002c;46:2847-56.

Gauldie J, Richards C, Baumann H. IL6 and the aucte phase reaction. Res Immunol 1992;143:755-9.

Geerts A. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. Semin Liver Dis 2001;21:311-35.

Geertsen P, Johansen JS, von der Maase H, Jensen BV, Price PA. High pretreatment serum level of YKL-40 is related to short survival in patients with advanced renal cell carcinoma treated with high-dose continuous intravenous infusion of interleukin-2. Meeting Proceedings of ASCO 2003;22:abstract 1603.

Goetzl EJ, Banda MJ, Leppert D. Matrix metallloproteinases in immunity. J Immunol 1996;156:1-4.

Goldberg RL, Huff JP, Lenz ME, Glickman P, Katz R, Thonar EJ-MA. Elevated plasma levels of hyaluronan in patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 1991;34:799-807.

Goronzy JJ, Matteson EL, Fulbright JW, Warrington KJ, Cang-Miller A, Hunder GG, Mason TG, Nelson AM, Valente RM, Crowson CS, Erlich HA, Reynolds RL, Swee RG, O'Fallon WM, Weyand CM. Prognostic markers of radiographic progression in early rheumatoid arthritis. Arthritis Rheum 2004;50:43-54.

Graudal NA, Jurik AG, de Carvalho A, Graudal HK. Radiographic progression in rheumatoid arthritis: a long-term prospective study of 109 patients. Arthritis Rheum 1998; 41:1470-80.

Graudal NA, Madsen HO, Tarp U, Svejgaard A, Jurik AG, Graudal HK, Garred P. The association of variant mannose-binding lectin genotypes

with radiographic outcome in rheumatoid arthritis. Arthritis Rheum 2000a;43:515-21.
Graudal NA, Tarp U, Jurik AG, Galløe AM, Garred P, Milman N, Graudal HK. Inflammatory patterns in rheumatoid arthritis estimated by the number of swollen and tender joints, the erythrocyte sedimentation rate, and hemoglobin: longterm course and association to radiographic progression. J Rheumatol 2000b;27:47-57.
Gravallese EM, Goldring SR. Cellular mechanisms and the role of cytokines in bone erosions in rheumatoid arthritis. Review. Arthritis Rheum 2000;43:2143-51.
Gravanis MB. Giant cell arteritis and Takayasu aortitis: morphologic, pathogenetic and etiologic factors. Int J Cardiol 2000;75 Suppl 1:S21-33.
Green M, Marzo-Ortega H, McGonagle D, Wakefield R, Proudman S, Conaghan P, Gooi J, Emery P. Persistence of mild, early inflammatory arthritis: the importance of disease duration, rheumatoid factor, and the shared epitope. Arthritis Rheum 1999;42:2184-8.
Gregoire M, Lieubeau B. The role of fibroblasts in tumor behavior. Cancer Metas Rev 1995;14:339-50.
Guoping C, Fan P, Jingxi S, Xiaoping L, Shiqin J, Yuri L. Purification and characterization of a silica-induced bronchoalveolar lavage protein with fibroblast growth-promoting activity. J Cell Biochem 1997;67:257-64.
Gøtzsche PC, Hansen M, Stoltenberg M, Svendsen A, Beier J, Faarvang KL, Wangel M, Rydgren L, Halberg P, Junker P, Andersen V, Hansen TM, Endahl L. Randomized, placebo controlled trial of withdrawal of slow-acting antirheumatic drugs and of observer bias in rheumatoid arthritis. Scand J Rheumatol 1996;25:194-9.
Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. J Biol Chem 1993;268:25803-10.
Hansen M, Johansen JS, Stoltenberg M, Gøtzsche PC, Svendsen A, Lorenzen I. Changes in biochemical markers of connective tissue metabolism in patients with rheumatoid arthritis after withdrawal of DMARD therapy. Scand J Rheum Dis, to be submitted.
Harada M, Mitsuyama K, Yoshida H, Sakisaka S, Taniguchi E, Kawaguchi T, Ariyoshi M, Saiki T, Sakamoto M, Nagata K, Sata M, Matsuo K, Tanikawa K. Vascular endothelial growth factor in patients with rheumatoid arthritis. Scand J Rheumatol 1998;27:377-80.
Haraoui B, Pelletier J-P, Cloutier J-M, Faure M-P, Martel-Pelletier J. Synovial membrane histology and immunopathology in rheumatoid arthritis and osteoarthritis: in vivo effects of antirheumatic drugs. Arthritis Rheum 1991;34: 153-63.
Harbord M, Novelli M, Canas B, Power D, Davis C, Godovac-Zimmermann J, Roes J, Segal AW. Ym1 is a neutrophil granule protein that crystallizes in $p47^{phox}$-deficient mice. J Biol Chem 2002;277:5468-75.
Harper PH, Fazio VW, Lavery IC et al. The long-term outcome in Crohn's disease. Dis Colon Rectum 1987;30:174-9.
Harris AL. Hypoxia – a key regulatory factor in tumour growth. Nature Reviews, Cancer 2002;2:38-47.
Harrison BJ, Symmons DPM, Brennan P, Barrett EM, Silman AJ. Natural remission in inflammatory polyarthritis: issues of definition and prediction. Br J Rheumatol 1996a;35: 1096-100.
Harrison BJ, Symmons DPM, Brennan P, Bankhead CR, Barrett EM, Scott DGI. Inflammatory polyarthritis in the community is not a benign disease: predicting functional disability one year after presentation. J Rheumatol 1996b; 23:1326-31.
Harrison BJ, Thomson W, Symmons D, Ollier B, Wiles N, Payton T et al. The influence of HLA-DRB1 alleles and rheumatoid factor on disease outcome in an inception cohort of patients with early inflammatory arthritis. Arthritis Rheum 1999;42:2174-83.
Harrison NK, Myers AR, Corrin B, Soosay G, Dewar A, Black CM, Du Bois RM, Turner-Warwick M. Structural features of intestinal lung disease in systemic sclerosis. Am Rev Respir Dis 1991;144:706-13.
Harvey S, Weisman M, O'Dell J, Scott T, Krusemeier M, Visor J, Swindlehurst C. Chondrex: new marker of joint disease. Clin Chem 1998;44:509-16.
Harvey S, Whaley J, Eberhardt K. The relationship between serum levels of YKL-40 and disease progression in patients with early rheumatoid arthritis. Scand J Rheumatol 2000; 29:391-3.
Hashimoto S, Setareh M, Ochs RL, Lotz M. Fas/Fas ligand expression and induction of apoptosis in chondrocytes. Arthritis Rheum 1997;40:1749-55.
Hashimoto S, Suzuki T, Dong H-Y, Yamazaki N, Matsushima K. Serial analysis of gene expression in human monocytes and macrophages. Blood 1999a;94:837-44.
Hashimoto S, Suzuki T, Dong H-Y, Nagai S, Yamazaki N, Matsushima K. Serial analysis of gene expression in human monocyte-derived dendritic cells. Blood 1999b;94:845-52.
Hassell AB, Davis MJ, Fowler PD, Clarke S, Fisher J, Shadforth ME, Jones PW, Dawes PT. The relationship between serial measures of disease activity and outcome in rheumatoid arthritis. QJM 1993;86:601-7.
Hassell AB, Plant MJ, Clarke S, Fisher J, Jones PW, Saklatvala J, Fowler PD, Shadforth MF, Dawes PT. Small joint synovitis in rheumatoid arthritis: should it be assessed separately? Br J Rheumatol 1995;34:51-5.
Haüselmann HJ, Aydelotte MB, Schumacker BL, Kuettner KE, Gitelis SH, Thonar EJ-MA. Synthesis and turnover of proteoglycans by human and bovine articular chondrocytes cultured in alginate beads. Matrix 1992;12: 116-29.
Haüselmann HJ, Fernandes RJ, Mok SS, Schmid TM, Block JA, Aydelotte MB, Kuettner K, Thonar EJ-MA. Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads. J Cell Sci 1994;107:17-27.
Haüselmann HJ, Flechtenmacher J, Michal L, Thonar EJ-MA, Shinmei M, Kuettner KE, Aydelotte MB. The superficial layer of human articular cartilage is more susceptible to interleukin-1-induced damage than the deeper layers. Arthritis Rheum 1996;39:478-88.
Hayes DF, Bast RC, Desch CE, Fritsche H, Kemeny NE, Jessup JM, Locker GY, Macdonald JS, Mennel RG, Norton L, Ravdin P, Taube S, Winn RJ. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996;88:1456-66.
Hayes DF. Determination of clinical utility of tumor markers: a tumor marker utility grading system. Recent Results Cancer Res 1998;152:71-85.
Hedin P-J, Weitoft T, Hedin H, Engström-Laurent A, Saxne T. Serum concentrations of hyaluronan and proteoglycan in joint disease. Lack of association. J Rheumatol 1991;18: 1601-5.
Henrissat B, Bairoch A. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J 1993;293:781-8.
Henrissat B, Davies G. Structural and sequence-based classification of glycoside hydrolases. Curr Opin Struct Biol 1997; 7:637-44.
Heremans H, Billiau A, Cassiman JJ, Mulier JC, de Somer P. In vitro cultivation of human tumor tissues. II. Morphological and virological characterization of three cell lines. Oncology 1978;35:246-52.
Hickling P, Jacoby RK, Kirwan JR and The Arthritis and Rheumatism Council Low-Dose Glucocorticoid Study Group. Joint destruction after glucocorticoids are withdrawn in early rheumatoid arthritis. Br J Rheumatol 1998;37:930-6.
Hittelman AB, Purohit RS, Kane CJ. Update of staging and risk assessment for prostate cancer patients. Curr Opin Urol 2004;14:163-70.
Hochberg MC. Adult and juvenile rheumatoid arthritis: current epidemiologic concepts. Epidemiol Rev 1981;3:27-44.
Hollingsworth JW, Siegel ER, Creasy WA. Granulocyte survival in synovial exudate of patients with rheumatoid arthritis and other inflammatory joint disease. Yale J Biol Med 1967;39:289-96.
Holten-Andersen MN, Stephens RW, Nielsen HJ, Murphy G, Christensen IJ, Stetler-Stevenson W, Brünner N. High preoperative plasma tissue inhibitor of metalloproteinase-1 levels are associated with short survival of patients with colorectal cancer. Clin Cancer Res 2000;6:4292-9.
Holten-Andersen MN, Christensen IJ, Nielsen HJ, Stephens RW, Jensen V, Nielsen OH, Sørensen S, Overgaard J, Lilja H, Harris A, Murphy G, Brünner N. Total levels of tissue inhibitor of metalloproteinases 1 in plasma yield high diagnostic sensitivity and specificity in patients with colon cancer. Clin Cancer Res 2002;8:156-64.
Hori Y, Hu DE, Yasui K, Smither RL, Gresham GA, Fan TB. Differential effects of angiostatic steroids and dexamethasone on angiogenesis and cytokine levels in rat sponge implants. Br J Pharmacol 1996;118:1584-91.
Houssiau FA, Devogelaer J-P, van Damme J, Nagant de Deuxchaisnes C, van Snick J. Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides. Arthritis Rheum 1988;31:784-8.
Houston DR, Recklies AD, Krupa JC, van Aalten DMF. Structure and ligand-induced conformational change of the 39-kDa glycoprotein from human articular chondrocytes. J Biol Chem 2003;278:30206-12.
Hu B, Trinh K, Figueira WF, Price PA. Isolation and sequence of a novel human chondrocyte protein related to mammalian members of the chitinase protein family. J Biol Chem 1996;271:19415-20.
Huang Y, Prasad M, Lemon WJ, Hampel H, Wright FA, Kornacker K, LiVolsi V, Frankel W, Kloos RT, Eng C, Pellegata NS, de la Chapelle A. Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. Proc Natl Acad Sci USA 2001;98:15044-9.
Hunder GG, Bloch DA, Michel BA, Stevens MB, Arend WP, Calabrese LH, Edworthy SM, Ffauci AS, Leavitt RY, Lie JT, Lightfoot RW, Masi AT, McShane DJ, Mills JA, Wallace SL, Zvaifler NJ. The american college of rheumatology 1990 criteria for the classification of giant cell arteritis. Arthritis Rheum 1990;33:1122-8.
Hunder GG, Lie JT, Goronzy JJ, Weyand CM. Pathogenesis of giant cell arteritis. Arthritis Rheum 1993;36:757-61.
Hulsmans HMJ, Jacobs JWG, van der Heijde DMFM, van Albada-Kuipers GA, Schenk Y, Bijlsma JWJ. The course of radiologic damage during the first six years of rheumatoid arthritis. Arthritis Rheum 2000;43:1927-40.
Høgdall EVS, Høgdall CK, Tingulstad S, Hagen B, Nustad K, Xu F-J, Bast RC, Jacobs IJ. Predictive values of serum tumour markers tetranectin, OVX1, CASA and CA125 in patients with a pelvic mass. Int J Cancer 2000a;89:519-23.
Høgdall EVS, Johansen JS, Kjaer SK, Price PA, Blaakjaer J, Høgdall CK.

Stability of YKL-40 concentration in blood samples. Scand J Clin Lab Invest 2000b;60:247-52.
Høgdall EVS, Johansen JS, Kjaer SK, Price PA, Christensen L, Blaakaer J, Bock JE, Glud E, Høgdall CK. High plasma YKL-40 level in patients with ovarian cancer stage III is related to shorter survival. Oncol Rep 2003;10:1535-8.
Hørslev-Petersen K, Bentsen KD, Junker P, Lorenzen I. Serum amino-terminal type III procollagen peptide in rheumatoid arthritis. Relationship to disease activity, treatment, and development of joint erosions. Arthritis Rheum 1986; 26:592-9.
Hørslev-Petersen K. Circulating extracellular matrix components as markers for connective tissue response to inflammation. A clinical and experimental study with special emphasis on serum aminoterminal type III procollagen peptide in rheumatic diseases. Thesis. Lægeforeningens forlag, København 1990.
Imabayashi H, Mori T, Gojo S, Kioyono T, Sugiyama T, Irie R, Isoga T, Hata J, Toyama Y, Umezawa A. Redifferentiation of dedifferentiated chondrocytes and chondrogenesis of human bone marrow stromal cells via chondrosphere formation with expression profiling by large-scale cDNA analysis. Exp Cell Res 2003;288:35-50.
Iozzo RV, Cohen IR, Grässel S, Murdoch AD. The biology of perlecan: the multifaceted heparan sulphate proteoglycan of basement membranes and pericellular matrices. Biochem J 1994;302:625-39.
Ip WK, Lau YL, Chan SY, Mok CC, Chan D, Tong KK, Lau CS. Mannose-binding lectin and rheumatoid arthritis in southern Chinese. Arthritis Rheum 2000;43:1679-87.
Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, Hovell C, Arthur MJP. Mechanisms of spontaneous resolution of rat liver fibrosis: hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest 1998;102:538-49.
Iredale JP. Hepatic stellate cell behaviour during resolution of liver injury. Semin Liver Dis 2001;21:427-36.
Ishiguro N, Ito T, Obata K-I, Fujimoto N, Iwata H. Determination of stromelysin-1, 72 and 92 kDa type IV collagenase, tissue inhibitor of metalloproteinase-1 (TIMP-1), and TIMP-2 in synovial fluid and serum from patients with rheumatoid arthritis. J Rheumatol 1996;23:1599-604.
Jensen BV, Johansen JS, Price PA. High levels of serum HER-2/neu and YKL-40 independently reflect aggressiveness of metastatic breast cancer. Clin Cancer Res 2003;9:4423-34.
Jin HM, Copeland NG, Gilbert DJ, Jenkins NA, Kirkpatrick RB, Rosenberg M. Genetic characterization of the murine Ym1 gene and identification of a cluster of highly homologous genes. Genomics 1998;54:316-22.
Johansen JS, Williamson MK, Rice JS, Price PA. Identification of proteins secreted by human osteoblastic cells in culture. J Bone Miner Res 1992;7: 501-12.
Johansen JS, Jensen HS, Price PA. A new biochemical marker for joint injury. Analysis of YKL-40 in serum and synovial fluid. Br J Rheumatol 1993;32: 949-55.
Johansen JS, Cintin C, Jørgensen M, Kamby C, Price PA. Serum YKL-40: a new potential marker of prognosis and location of metastases of patients with recurrent breast cancer. Eur J Cancer 1995;31A:1437-42.
Johansen JS, Hvolris J, Hansen M, Backer V, Lorenzen I, Price PA. Serum YKL-40 levels in healthy children and adults. Comparison with serum and synovial fluid levels of YKL-40 in patients with osteoarthritis or trauma of the knee joint. Br J Rheumatol 1996a;35:553-9.
Johansen JS, Stoltenberg M, Hansen M, Hansen TM, Price PA, Gøtzsche PC. Serum YKL-40 reflects disease relapse after withdrawal of SAARD in patients with rheumatoid arthritis. Arthritis Rheum 1996b;39(Suppl): abstract 768.
Johansen JS, Møller S, Price PA, Bendtsen F, Junge J, Garbarsch C, Henriksen JH. Plasma YKL-40: a new potential marker of fibrosis in patients with alcoholic cirrhosis? Scand J Gastroenterol 1997;32:582-90.
Johansen JS, Baslund B, Garbarsch C, Hansen M, Stoltenberg M, Lorenzen I, Price PA. YKL-40 in giant cells and macrophages from patients with giant cell arteritis. Arthritis Rheum 1999a;42:2624-30.
Johansen JS, Stoltenberg M, Hansen M, Florescu A, Hørslev-Petersen K, Lorenzen I, Price PA. Serum YKL-40 concentrations in patients with rheumatoid arthritis: relation to disease activity. Rheumatology 1999b;38:618-26.
Johansen JS, Christoffersen P, Møller S, Price PA, Henriksen JH, Garbarsch C, Bendtsen F. Serum YKL-40 is increased in patients with hepatic fibrosis. J Hepatol 2000a;32:911-20.
Johansen JS, Klarlund M, Price PA, Jensen KE, Skjødt H and the TIRA group. Serum YKL-40 levels in patients with unclassified polyarthritis and early rheumatoid arthritis. Arthritis Rheum 2000b;43(Suppl): abstract 533.
Johansen JS, Hansen M, Klarlund M, Price PA, Volck B, Lorenzen I, DRD1 group and TIRA group. Serum YKL-40 levels in patients with rheumatoid arthritis. Arthritis Rheum 2001a;44(Suppl): abstract 1355.
Johansen JS, Kirwan JR, Price PA, Sharif M. Serum YKL-40 concentrations in patients with early rheumatoid arthritis: relation to joint destruction. Scand J Rheumatol 2001b;30: 297-304.
Johansen JS, Olee T, Price PA, Hashimoto S, Ochs RL, Lotz M. Regulation of YKL-40 production by human articular chondrocytes. Arthritis Rheum 2001c;44:826-37.
Johansen JS, Christensen IJ, Riisbro R, Greenall M, Han C, Price PA, Smith K, Brünner N, Harris AL. High serum YKL-40 levels in patients with primary breast cancer is related to short recurrence free survival. Breast Cancer Res Treatment 2003a;80:15-21.
Johansen JS, Hetland ML, Østergaard M, Majgaard O, Skjødt H. Changes in serum YKL-40 levels in patients with rheumatoid arthritis and ankylosing spondylitis during treatment with anti-TNF-α therapy. Arthritis Rheum 2003b;48(Suppl): abstract 1393.
Johansen JS, Drivsholm L, Price PA, Christensen IJ. High serum YKL-40 level in patients with small cell lung cancer is related to early death. Lung Cancer 2004;46:333-40.
Johansen JS, Krabbe K, Møller K, Pedersen BK. Circulating YKL-40 levels during human endotoxaemia. Clin Exp Immunol 2005a;140:343-8.
Johansen JS, Milman N, Hansen M, Garbarsch C, Price PA, Graudal N. Increased serum YKL-40 in patients with pulmonary sarcoidosis. A potential marker of disease activity? Resp Med 2005b;99:396-402.
Joosten LAB, Coenen de-Roo CJJ, Helsen MMA, Lubberts E, Boots AMH, van den Berg WB, Miltenburg AMM. Induction of tolerance with intranasal administration of human cartilage gp-39 in DBA/1 mice. Arthritis Rheum 2000;43: 645-55.
Junker N, Johansen JS, Andersen CB, Kristjansen PEG. Expression of YKL-40 by peritumoral macrophages in human small cell lung cancer. Lung Cancer 2005a;48:223-31.
Junker N, Johansen JS, Hansen LT, Lund EL, Kristjansen PEG. Regulation of YKL-40 expression during genotoxic or microenvironmental stress in human glioblastoma cells. Cancer Science 2005b;96:183-90.
Kammermann JR, Kincaid SA, Rumph PF, Baird DK, Visco DM. Tumor necrosis factor-α (TNF-α) in canine osteoarthritis: immunolocalization of TNF-α, stromelysin and TNF receptors in canine osteoarthritic cartilage. Osteoarthritis Cartilage 1996;4:23-34.
Kavanaugh A, Genovese M, Baughman J, Kivitz A, Bulpitt K, Olsen N, Weisman M, Matteson E, Furst D, van Vollenhoven R, Anderson J, Cohen S, Wei N, Meijerink J, Jacobs C, Mocci S. Allele and antigen-specific treatment of rheumatoid arthritis: a double blind, placebo controlled phase 1 trial. J Rheumatol 2003;30:449-54.
Kawamura K, Shibata T, Saget O, Peel D, Bryant PJ. A new family of growth factors produced by the fat body and active on Drosophila imaginal disc cells. Development 1999; 126:211-9.
Kawanaka N, Yamamura M, Aita T, Morita Y, Okamoto A, Kawashima M, Iwahashi M, Ueno A, Ohmoto Y, Makino H. CD14+,CD16+ blood monocytes and joint inflammation in rheumatoid arthritis. Arthritis Rheum 2002;46: 2578-86.
Kawasaki M, Hasegawa Y, Kondo S, Iwata H. Concentration and localization of YKL-40 in hip joint diseases. J Rheumatol 2001;28:341-5.
Kenny PA, Bissell MJ. Tumor reversion: correction of malignant behavior by microenvironmental cues. Int J Cancer 2003;107:688-95.
Keyszer G, Lambiri I, Nagel R, Keysser C, Keysser M, Gromnica-Ihle E, Franz J, Burmester GR, Jung K. Circulating levels of matrix metalloproteinases MMP-3 and MMP-1, tissue inhibitor of metalloproteinases 1 (TIMP-1), and MMP-1/TIMP-1 complex in rheumatic disease. Correlation with clinical activity of rheumatoid arthritis versus other surrogate markers. J Rheumatol 1999;26:251-8.
Khan MA. Update: the twenty subtypes of HLA-B27. Curr Opin Rheumatol 2000;12:235-8.
Khan MA, Partin AW. Management of patients with an increasing prostate-specific antigen after radical prostatectomy. Curr Urol Rep 2004;5:179-87.
Kirkpatrick RB, Matico RE, McNulty DE, Strickler JE, Rosenberg M. An abundantly secreted glycoprotein from Drosophila melanogaster is related to mammalian secretory proteins produced in rheumatoid tissues and by activated macrophages. Gene 1995;153:147-54.
Kirkpatrick RB, Emery JG, Connor JR, Dodds R, Lysko PG, Rosenberg M. Induction and expression of human cartilage glycoprotein 39 in rheumatoid inflammatory and peripheral blood monocyte-derived macrophages. Exp Cell Res 1997;237:46-54.
Kirsch AH, Mahmood AA, Endres J, Bohra L, Bonish B, Weber K, Fox DA. Apoptosis of human T-cells: induction by glucocorticoids or surface receptor ligation in vitro and ex vivo. J Biol Regul Homeost Agents 1999;13:80-9.
Kirwan JR and the ACR Low Dose Glucocorticoid Study Group. The effect of glucocorticoids on joint destruction in rheumatoid arthritis. N Engl J Med 1995;333:142-6.
Kirwan JR. The relationship between synovitis and erosions in rheumatoid arthritis. Br J Rheumatol 1997;36:225-8.
Kirwan JR, Byron M, Watt I. The relationship between soft tissue swelling, joint space narrowing and erosive damage in hand X-rays of patients with rheumatoid arthritis. Rheumatology 2001;40:297-301.
Kirwan JR. The synovium in rheumatoid arthritis: evidence for (at least) two pathologies. Arthritis Rheum 2004;50:1-4.
Klarlund M, Brünner N, Nielsen HJ, Johansen JS, Hansen M, Skjødt H, Madsen RR, Jacobsen S and the DRD1 and TIRA group. Circulating

markers of angiogenesis and plasminogen activation system in patients with rheumatoid arthritis. Arthritis Rheum 2000;43(Suppl): abstract 721.

Knorr T, Obermayr F, Bartnik E, Zien A, Aigner T. YKL-39 (chitinase 3-like protein 2), but not YKL-40 (chitinase 3-like protein 1), is up regulated in osteoarthritic chondrocytes. Ann Rheum Dis 2003;62:995-8.

Koch AE, Harlow LA, Haines GK, Amento EP, Unemori EN, Wong WL, Pope RM, Ferrara N. Vascular endothelial growth factor. A cytokine modulating endothelial function in rheumatoid arthritis. J Immunol 1994;152: 4149-56.

Koch AE. Angiogenesis. Implications for rheumatoid arthritis. Arthritis Rheum 1998;41:951-62.

Koch AE. The role of angiogenesis in rheumatoid arthritis: recent developments. Ann Rheum Dis 2000;59(Suppl I): i65-i71.

Konttinen YT, Li TF, Hukkanen M, Ma J, Xu JW, Virtanen I. Fibroblast biology. Signals targeting the synovial fibroblast in arthritis. Arthritis Res 2000;2:348-55.

Kosher RA, Kulyk WM, Gay SW. Collagen gene expression during limb cartilage differentiation. J Cell Biol 1986;102: 1151-6.

Koutroubakis IE, Petinaki E, Dimoulios P, Vardas E, Roussomoustakaki M, Maniatis AN, Kouroumalis EA. Increased serum levels of YKL-40 in patients with inflammatory bowel disease. Int J Colorectal Dis 2003;18: 254-9.

Kotake S, Sato K, Kim KJ, Takahashi N, Udagawa N, Nakamura I, Yamaguchi A, Kishimoto T, Suda T, Kashiwazaki S. Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. J Bone Miner Res 1996;11:88-95.

Krabbe K, Bruunsgaard H, Hansen CM, Møller K, Fonsmark L, Qvist J, Lau PM, Kronborg G, Andersen HØ, Skinhøj P, Pedersen BK. Ageing is associated with a prolonged fever response in human endotoxemia. Clin Diag Lab Immunol 2001;8:333-8.

Kraan MC, de Koster BM, Elferink JG, Post WJ, Breedveld FC, Tak PP. Inhibition of neutrophil migration soon after initiation of treatment with leflunomide or methotrexate in patients with rheumatoid arthritis: findings in a prospective, randomized, double-blind clinical trial in fifteen patients. Arthritis Rheum 2000;43:1488-95.

Krause SW, Rehli M, Kreutz M, Schwarzfisher L, Paulauski JD, Andreesen R. Differential screening identifies genetic markers of monocyte to macrophage maturation. J Leukocyte Biol 1996;60:540-5.

Kronborg G, Østergaard C, Weis N, Nielsen H, Obel N, Pedersen SS, Price PA, Johansen JS. Serum level of YKL-40 is elevated in patients with Streptococcus pneumoniae bacteremia and is associated with the outcome of the disease. Scand J Infect Dis 2002;34:323-6.

Kushner I. The phenomenon of the acute phase response. Ann NY Acad Sci 1982;389:38-48.

Kushner I. C-reactive protein in rheumatology. Arthritis Rheum 1991;34: 1065-8.

Lal A, Lash AE, Altschul SF, Velculescu V, Zhang L, McLendon RE, Marra MA, Prange C, Morin PJ, Polyak K, Papadopoulos N, Vogelstein B, Kinzler KW, Strausberg RL, Riggins GJ. A public database for gene expression in human cancers. Cancer Res 1999;59:5403-7.

Landewe RBM, Boers M, Verhoeven AC, Westhovens R, van de Laar MAFJ, Markusse HM, van Denderen JC, Westedt ML, Peeters AJ, Dijkmans BAC, Jacobs P, Boonen A, van der Heijde DMFM, van der Linden S. COBRA combination therapy in patients with early rheumatoid arthritis. Long-term structural benefits of a brief intervention. Arthritis Rheum 2002;46:347-56.

Laurent TC. The chemistry, biology and medical applications of hyaluronan and its derivatives. Wenner-Gren International Series. 2001;72: Portland Press. London and Miami.

Lawrance IC, Fiocchi C, Charkravarti S. Differential expression of metalloproteinases (MMPs), tissue inhibitor of metalloproteinases (TIMP)-1 and extracellular matrix (ECM) components in fibrosed, inflamed and normal intestine. Gastroenterol 1999;116:A759.

Lawrance IC, Maxwell L, Doe W. Inflammation location, but not type, determines the increase in TGF-β1 and IGF-1 expression and collagen deposition in IBD intestine. Inflamm Bowel Dis 2001;7:16-26.

Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, Heyse SP, Hirsch R, Hochberg MC, Hunder GG, Liang MH, Pillemer SR, Steen VD, Wolfe F. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum 1998;41: 778-99.

Lee JY, Spicer AP. Hyaluronan: a multifunctional, megaDalton, stealth molecule. Curr Opin Cell Biol 2000;12:581-6.

Levy AP, Levy NS, Goldberg MA. Post-transcriptional regulation of vascular endothelial growth factor by hypoxia. J Biol Chem 1996;271:2746-53.

Li D, Friedman SL. Liver fibrogenesis and the role of hepatic stellate cells: new insights and prospects for therapy. J Gastroenterol Hepatol 1999;14: 618-33.

Lieberman J, Schlessner LA, Nosal A, Sastre A, Mishkin FS. Clinical correlations of serum angiotensin-converting enzymes (ACE) in sarcoidosis. A longitudinal study of serum ACE, gallium-67 scans, ches roentgenograms and pulmonary function. Chest 1983;84:522-8.

Lin EY, Nguyen AV, Russell RG, Pollard JW. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med 2001;193:727-39.

Lin EY, Pollard JW. Macrophages: modulators of breast cancer progression. In: Cancer and Inflammation. Norvatis foundation Symposium 256. Wiley, Chichester. 2004a, p 156-172.

Lin EY, Pollard JW. Role of infiltrated leucocytes in tumour growth and spread. Br J Cancer 2004b;90:2053-8.

Lindahl U, Kusche-Gullberg M, Kjellen L. Regulated diversity of heparan sulfate. J Biol Chem 1998;273:24979-82.

Ling H, Recklies AD. The chitinase 3-like protein human cartilage glycoprotein 39 inhibits cellular responses to the inflammatory cytokines interleukin-1 and tumour necrosis factor-alpha. Biochem J 2004;380:651-9.

Lotz M, Blanco FJ, von Kepmis J, Dudler J, Maier R, Villiger PM, Geng Y. Cytokine regulation of chondrocyte functions. J Rheumatol 1995;22 (Suppl 43):104-8.

Lunevicius R, Nakanishi H, Ito S, Kozaki K-I, Kato T, Tatematsu M, Yasui K. Clinicopathological significance of fibrotic capsule formation around liver metastasis form colorectal cancer. J Cancer Res Clin Oncol 2001;127:193-9.

Maciel SB, Scheinberg MA. Serum chondrex values in knee osteoarthritis (OA). The effect of arthroscopy. Clin Rheumatol 2000;19:76-7.

MacNaul NI, Chartrain N, Lark M, Tocci MJ, Hutchinson NI. Discoordinate expression of stromelysin, collagenase, and tissue inhibitor of metalloproteinases-1 in rheumatoid human synovial fibroblasts. Synergistic effects of interleukin-1 and tumor necrosis factor-α on stromelysin expression. J Biol Chem 1990;265:17238-45.

MacNee W, Selby C. Neutrophil kinetics in the lungs. Clin Sci 1990;79:97-107.

Mahaney MC, Czerwinski SA, Rogers J. Genetic effects on serum levels of human cartilage glycoprotein-39 (YKL-40) in a pedigreed baboon model for age-related changes and pathology in bone. Am Soc Bone Miner Res 1998; Abstract no. SA121.

Maher JJ. Cytokines: overview. Semin Liver Dis 1999;19:109-15

Maksymowych WP, Jhangri GS, Lambert RG, Mallon C, Buenviaje H, Pedrycz E, Luongo R, Russell AS. Infliximab in ankylosing spondylitis: a prospective observational inception cohort analysis of efficacy and safety. J Rheumatol 2002;29:959-65.

Malette B, Paquette Y, Merlen Y, Bleu G. Oviductin possess chitinase- and mucin-like domains: a lead in the search for the biological function of these oviduct-specific ZP-associating glycoproteins. Mol Reprod Dev 1995;41:384-97.

Malinda KM, Ponce L, Kleinman HK, Shackelton LM, Millis AJT. Gp38k, a protein synthesized by vascular smooth muscle cells, stimulates directional migration of human umbilical vein endothelial cells. Exp Cell Res 1999;250: 168-73.

Manicourt D-H, Triki R, Fukuda K, Devogelaer J-P, Nagant de Deuxchaisnes C, Thonar EJMA. Levels of circulating tumor necrosis factor α and interleukin-6 in patients with rheumatoid arthritis. Arthritis Rheum 1993;36:490-9.

Mapp PI, Grootveld MC, Blake DR. Br Med Bull 1995;51: 419-36.

Markert JM, Fuller CM, Gillespie GY, Bubien JK, McLean LA, Hong RL, Lee K, Gullans SR, Mapstone TB, Benos DJ. Differential gene expression profiling in human brain tumors. Physiol Genomics 2001;5:21-33.

Marti M, Alvarez I, Lopez de Castro JA. A molecular insight on the association of HLA-B27 with spondylo-arthropathies. Curr Rheumatol Rep 1999;1:78-85.

Matsumoto T, Tsurumoto T. Serum YKL-40 levels in rheumatoid arthritis: correlations between clinical and laboratory parameters. Clin Exp Rheumatol 2001;19:655-60.

Matthes H, Herbst H, Schuppan D, Stallmach A, Milani S, Stein H, Riecken E-O. Cellular localization of procollagen gene transcripts in inflammatory bowel diseases. Gastroenterology 1992;102:431-42.

McGonagle D, Conaghan PG, O'Connor P, Gibbon W, Green M, Wakefield R, Ridgway J, Emery P. The relationship between synovitis and bone changes in early untreated rheumatoid arthritis. Arthritis Rheum 1999;42:1706-11.

McLeod HL, Murray GI. Tumor markers of prognosis in colorectal cancer. Br J Cancer 1999;79:191-203.

Meyer MF, Kreil G. Cells expressing the DG42 gene from early Xenopus embryos synthesize hyaluronan. Proc Natl Acad Sci USA 1996;93:4543-7.

Millis AJT, Hoyle M, Reich E, Mann DM. Isolation and characterization of a Mr = 38,000 protein from differentiating smooth muscle cells. J Biol Chem 1985;260:3754-61.

Millis AJT, Hoyle M, Kent L. In vitro expression of a 38,000 dalton heparin-binding glycoprotein by morphologically differentiated smooth muscle cells. J Cell Physiol 1986;127: 366-72.

Milman N, Selroos O. Pulmonary sarcoidosis in the Nordic countries 1950-1982. I. Epidemiology and clinical picture. Sarcoidosis 1990a;7:50-7.

Milman N, Selroos O. Pulmonary sarcoidosis in the Nordic countries 1950-1982. II. Course and prognosis. Sarcoidosis 1990b;7:113-8.
Miossec P. Interleukin-17 in rheumatoid arthritis. If T cells were to contribute to inflammation and destruction through synergy. Review. Arthritis Rheum 2003;48:594-601.
Mitchell EP. Role of carcinoembryonic antigen in the management of advanced colorectal cancer. Semin Oncol 1998; 25(Suppl 11):12-20.
Mitsiades CS, Mitsiades N, Koutsilieris M. Curr Cancer Drug Targets 2004;4:235-56.
Mohanty AK, Singh G, Paramasivam M, Saravanan K, Jabeen T, Sharma S, Yadav S, Kaur P, Kumar P, Srinivasan A, Singh TP. Crystal structure of a novel regulatory 40-kDa mammary gland protein (MGP-40) secreted during involution. J Biol Chem 2003;278:14451-60.
Molenaar ETH, Voskuyl AE, Dinant HJ, Bezemer PD, Boers M, Dijkmans BAC. Progression of radiologic damage in patients with rheumatoid arthritis in clinical remission. Arthritis Rheum 2004;50:36-42.
Montagna GL, D'Angelo S, Valentini G. Cross-sectional evaluation of YKL-40 serum concentrations in patients with systemic sclerosis. Relationship with clinical and serological aspects of disease. J Rheumatol 2003;30:2147-51.
Moore AR, Iwamura H, Larbre JP, Scott DL, Willoughby DA. Cartilage degradation by polymorphonuclear leucocytes: in vitro assessment of the pathogenic mechanisms. Ann Rheum Dis 1993;52:27-31.
Moreland LW, O'Dell JR. Glucocorticoids and rheumatoid arthritis. Arthritis Rheum 2002;46:2553-63.
Morrissette N, Gold E, Aderem A. The macrophage – a cell for all seasons. Trends Cell Biol 1999;9:199-201.
Morrison BW, Leder P. neu and ras initiate murine mammary tumors that share genetic markers generally absent in c-myc and int-2-initiated tumors. Oncogene 1994;9:3417-26.
Möttönen T, Paimela L, Leirisalo-Repo M, Kautiainen H, Ilonen J, Hannonen P. Only high disease activity and positive rheumatoid factor indicate poor prognosis in patients with early rheumatoid arthritis treated with "sawtooth" strategy. Ann Rheum Dis 1998;57:533-9.
Mueller A, O'Rourke J, Grimm J, Guillemin K, Dixon MF, Lee A, Falkow S. Distinct gene expression profiles characterize the histopathological stages of disease in Helicobacter-induced mucosa-associated lymphoid tissue lymphoma. Proc Natl Acad Science USA 2003;100:1292-7.
Mulherin D, FitzGerald O, Bresnihan B. Clinical improvement and radiological deterioration in rheumatoid arthritis: evidence that the pathogenesis of synovial inflam- mation and articular erosion may differ. Br J Rheumatol 1996a;35:1263-8.
Mulherin D, FitzGerald O, Bresnihan B. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum 1996b;39:115-24.
Munkholm P, Langholz E, Davidsen M, Binder V. Disease activity courses in a regional cohort of Crohn's disease patients. Scand J Gastroenterol 1995;30:699-706.
Myers SL. Synovial fluid markers in osteoarthritis. Rheum Dis Clin North AM 1999;25:433-49.
Månsson B, Carey D, Alini M, Ionescu M, Rosenberg LC, Poole AR, Heinegård D, Saxne T. Cartilage and bone metabolism in rheumatoid arthritis. J Clin Invest 1995;95: 1071-7.
Nagase H, Woessner JF. Matrix metalloproteinases. J Biol Chem 1999;274: 21491-4.
Nakamura H, Yoshino S, Kato T, Tsuruha J, Nishioka K. T-cell mediated inflammatory pathway in osteoarthritis. Osteoarthritis Cartilage 1999;7: 401-2.
Nathan CF. Secretory products of macrophages. J Clin Invest 1987;79:319-26.
Neaud V, Faouzi S, Guirouilh J, Le Bail B, Balabaud C, Bioulac-Sage P, Rosenbaum J. Human hepatic myofibroblasts increase invasiveness of hepatocellular carcinoma cells: evidence for a role of hepatocyte growth factor. Hepatology 1997;26:1458-66.
Nelson S, Mason CM, Kolls J, Summer WR. Pathophysiology of pneumonia. Clin Chest Med 1995;16:1-12.
Nepom GT, Gersuk V, Nepom BS. Prognostic implications of HLA genotyping in the early assessment of patients with rheumatoid arthritis. J Rheumatol 1996;23(Suppl 44):5-9.
Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 1999;13:9-22.
Newman, LS, Rose CS, Maier LA. Sarcoidosis. N Engl J Med 1997;336:1224-34.
Nishikawa KC, Millis AJT. Gp38k (CHI3L1) is a novel adhesion and migration factor for vascular cells. Exp Cell Res 2003;287:79-87.
Nishimoto N, Kishimoto T, Yoshizaki K. Anti-interleukin 6 receptor antibody treatment in rheumatic disease. Ann Rheum Dis 2000;59:i21-7.
Nord HJ. Biopsy diagnosis of cirrhosis: blind percutaneous versus guided direct vision techniques – a review. Gastrointest Endosc 1982;28:102-4.
Nordenbaek C, Johansen JS, Junker P, Borregaard N, Sørensen O, Price PA. YKL-40, a matrix protein of specific granules in neutrophils, is elevated in serum of patients with community-acquired pneumonia requiring hospitalization. J Infect Dis 1999;180:1722-6.
Nordenbæk C, Johansen JS, Halberg P, Wiik A, Garbarsch C, Ullman S, Price PA, Jacobsen S. High serum levels of YKL-40 in patients with systemic sclerosis are associated with pulmonary involvement. Scand J Rheumatol 2005;34:293-7.
Nuki G. Role of mechanical factors in the aetiology, pathogenesis and progression of osteoarthritis. In: Reginster JY, Pelletier JP, Martel-Pelletier J, Henrotin Y, editors. Osteoarthritis: clinical and experimental aspects. Berlin: Springer-Verlag 1999:101-14.
Nunes DP, Gwynneth DO, Keaveny A, Maldanado N, O'Brien M, Wilson S, Afdhal NH. Comparative study of YKL-40 (Chondrex), procollagen III peptide and hyaluronan for the diagnosis of hepatitis C associated liver disease. Hepatology 1998;28:abstract 984, p 408A.
Nyirkos P, Golds EE. Human synovial cells secrete a 39 kDa protein similar to a bovine mammary protein expressed during the non-lactating period. Biochem J 1990;268:265-8.
Nøjgaard C, Johansen JS, Christensen E, Skovgaard LT, Price PA, Becker U and The EMALD Group. Serum levels of YKL-40 and PIIINP as prognostic markers in patients with alcoholic liver disease. J Hepatol 2003a;39: 179-86.
Nøjgaard C, Johansen JS, Krarup HB, Holten-Andersen M, Møller A, Bendtsen F and the Danish Viral Hepatitis Study Group. Effect of antiviral therapy on markers of fibrogenesis in patients with chronic hepatitis C. Scand J Gastroenterol 2003b;38:659-65.
Oh S, Afdhal NH. Hepatic fibrosis: are any of the serum markers useful? Curr Gastroenterol Rep 2001;3:12-8.
Olaso E, Santisteban A, Bidaurrazaga J, Gressner AM, Rosenbaum J, Vidal-Vanaclocha F. Tumor-dependent activation of rodent hepatic stellate cells during experimental melanoma metastasis. Hepatology 1997;26:634-42.
Olaso E, Friedman SL. Molecular regulation of hepatic fibrogenesis. J Hepatol 1998;29:836-47.
Olee T, Hashimoto S, Quach J, Lotz M. IL-18 is produced by articular chondrocytes and induces proinflammatory and catabolic responses. J Immunol 1999;1096-100.
Olsen NJ, Stein CM. New drugs for rheumatoid arthritis. N Engl J Med 2004;350:2167-79.
Otterness IG. The value of C-reactive protein measurement in rheumatoid arthritis. Semin Arthritis Rheum 1994;24: 91-104.
Owen CA, Campbell EJ. The cell biology of leukocyte-mediated proteolysis. J Leukoc Biol 1999;65:137-50.
Owens GR, Paradis IL, Gryzan S, Medsger TA, Follansbee WP, Klein HA, Dauber JH. Role of inflammation in the lung disease of systemic sclerosis: comparison with idiopathic pulmonary fibrosis. J Lab Clin Med 1986;107: 253-60.
Owhashi M, Arita H, Hayai N. Identification of a novel eosinophil chemotactic cytokine (ECF-L) as a chitinase family protein. J Biol Chem 2000;275:1279-86.
Paimela L, Heiskanen A, Kurki P, Helve T, Leirisalo-Repo M. Serum hyaluronate level as a predictor of radiologic progression in early rheumatoid arthritis. Arthritis Rheum 1991;34:815-21.
Pando JA, Duray P, Yarboro C, Gourley MF, Klippel JH, Schumacher HR. Synovitis occurs in some clinically normal and asymptomatic joints in patients with early arthritis. J Rheumatol 2000;27:1848-54.
Paleolog EM, Miotla JM. Angiogenesis in rheumatoid arthritis: role in disease pathogenesis and as a potential therapeutic target. Angiogenesis 1998a;2:295-307.
Paleolog EM, Young S, Stark AC, McCloskey RV, Feldmann M, Maini RN. Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor α and interleukin-1 in rheumatoid arthritis. Arthritis Rheum 1998b; 41:1258-65.
Paleolog EM. Angiogenesis in rheumatoid arthritis. Arthritis Res 2002;4 (Suppl 3):s81-s90.
Partin AW, Kattan MW, Subong ENP, Walsh PC, Wojno KJ, Oesterling JE, Scardino PT, Pearson JD. Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer. A multi-institutional update. JAMA 1997;277:1445-51.
Patil NS, Hall FC, Drover S, Spurrell DR, Bos E, Cope AP, Sonderstrup G, Mellins ED. Autoantigenic HCgp39 epitopes are presented by the HLA-DM-dependent presentation pathway in human B cells. J Immunol 2001;166:33-41.
Pavelka K, Forejtova S, Olejarova M, Gatterova J, Senolt L, Spacek P, Braun M, Hulejova M, Stovickova J, Pavelkova A. Hyaluronic acid levels may have predictive value for the progression of knee osteoarthritis. Osteoarthritis Cartilage 2004;12:277-83.
Pearson D, Sheldon P. Effects of corticosteroid on lymphocyte adhesion. Adv Exp Med Biol 1995;371A:167-70.
Pelletier J-P, Martel-Pelletier J, Howell DS. Etiopathogenesis of osteoarthritis. In: Koopman WJ, editor. Arthritis & allied conditions: a textbool of rheumatology. 14th ed. Baltimore: Lippincott Williams & Wilkins 2000: 2195-245.
Pelletier J-P, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflamma-

tory disease. Potential implication for the selection of new therapeutic targets. Arthritis Rheum 2001; 44:1237-47.
Peltomaa R, Paimela L, Harvey S, Helve T, Leirisalo-Repo M. Increased level of YKL-40 in sera from patients with early rheumatoid arthritis: a new marker for disease activity. Rheumatol Int 2001;20:192-6.
Perrimon N, Bernfield M. Specificities of heparan sulphate proteoglycans in developmental processes. Nature 2000; 404:725-8.
Petersson IF, Boegård T, Svensson B, Heinegård D, Saxne T. Changes in cartilage and bone metabolism identified by serum markers in early osteoarthritis of the knee joint. Br J Rheumatol 1998;37:46-50.
Phillinger MH, Abramson SB. The neutrophil in rheumatoid arthritis. Rheum Dis Clin North Am 1995;21:691-714.
Piccinino F, Sagnelli E, Pasquale G, Giusti G. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. J Hepatol 1986;2:165-73.
Pinals RA, Masi AT, Larsen RA, and the Subcommittee for Criteria of Remission in Rheumatoid Arthritis of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee:. Preliminary criteria for clinical remission in rheumatoid arthritis. Arthritis Rheum 1981;24:1308-15.
Pinzani M. Liver fibrosis. Springer Semin Immunopathol 2000;21:475-90.
Plant MJ, Williams AL, O'Sullivan MM, Lewis PA, Coles EC, Jessop JD. Relationship between time-integrated C-reactive protein levels and radiologic progression in patients with rheumatoid arthritis. Arthritis Rheum 2000;43:1473-7.
Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nature Rev 2004;4:71-8.
Poole AR. Cartilage in health and disease. In: Koopman WJ, editor. Arthritis and allied conditions. Baltimore: Lippincott Williams and Wilkins 2003;226-84.
Poole AR. Can serum biomarker assays measure the progression of cartilage degeneration in osteoarthritis? Arthritis Rheum 2002;46:2549-52.
Posthumus MD, Limburg PC, Westra J, Cats HA, Stewart RE, van Leeuwen MA, van Rijswijk MH. Serum levels of matrix metalloproteinase-3 in relation to the development of radiological damage in patients with early rheumatoid arthritis. Rheumatology 1999;38:1081-7.
Poulsen H, Christoffersen P. Atlas of liver biopsies. Copenhagen: Munksgaard, Denmark, 1979.
Punzi L, Podswiadek M, D'Inca R, Zaninotto M, Bernardi D, Plebani M, Sturniolo GC. Serum human cartilage glycoprotein 39 as a marker of arthritis associated with inflammatory bowel disease. Ann Rheum Dis 2003;62:1224-6.
Qu Z, Hernandez GC, O'Rourke LM, Planck SR, Kohli M, Rosenbaum JT. Local proliferation of fibroblast-like synoviocytes contributes to synovial hyperplasia: results of proliferating cell nuclear antigen/cyclin, c-myc, and nucleolar organizer region staining. Arthritis Rheum 1994; 37: 212-20.
Rakic J-M, Lambert V, Deprez M, Foidart J-M, Noël A, Munaut C. Estrogens reduce the expression of YKL-40 in the retina: implications for eye and joint diseases. Invest Ophthalmol Vis Sci 2003;44:1740-6.
Reardon D, Farber GK. The structure and evolution of α/β barrel proteins. FASEB J 1995;9:497-503.
Recklies AD, White C, Ling H. The chitinase 3-like protein human cartilage glycoprotein 39 (HC-gp39) stimulates proliferation of human connective-tissue cells and activates both extracellular signal-regulated kinase- and protein kinase B-mediated signalling pathways. Biochem J 2002;365:119-26.
Reginato AM, Iozzo RV, Jimenez SA. Formation of nodular structures resembling mature articular cartilage in long-term primary cultures of human fetal epiphyseal chondrocytes on a hydrogel substrate. Arthritis Rheum 1994;37: 1338-49.
Register TC, Carlson CS, Adams MR. Serum YKL-40 is associated with osteoarthritis and atherosclerosis in nonhuman primates. Clin Chem 2001;47: 2159-61.
Rehli M, Krause SW, Andreesen R. Molecular characterization of the gene for human cartilage gp-39 (CHI3L1), a member of the chitinase protein family and marker for late stages of macrophage differentiation. Genomics 1997; 43:221-5.
Rehli M, Niller H-H, Ammon C, Langmann S, Schwarzfischer L, Andreesen R, Krause SW. Transcriptional regulation of *CHI3L1*, a marker gene for late stages of macro- phage differentiation. J Biol Chem 2003;278:44058-67.
Rejman JJ, Hurley WL. Isolation and characterization of a novel 39 kilodalton whey protein from bovine mammary secretions collected during the nonlactating period. Biochem Biophys Res Commun 1988;150:329-34.
Rejman JJ, Hurley WL, Bahr JM. Enzyme-linked immunosorbent assays of bovine lactoferrin and a 39-kilodalton protein found in mammary secretions during involution. J Dairy Sci 1989;72:555-60.
Remmers EF, Sano H, Wilder RL. Platelet-derived growth factors and heparin-binding (fibroblast) growth factors in the synovial pathology of rheumatoid arthritis. Semin Arthritis Rheum 1991;21:191-9.
Renkema GH, Boot RG, Au FL, Donker-Koopman WE, Strijland A, Muijsers AO, Hrebicek M, Aerts JMFG. Chitotriosidase, a chitinase, and the 39-kDa human cartilage glycoprotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrophages. Eur J Biochem 1998;251:504-9.
Ribbens C, Andre B, Jaspar J-M, Kaye O, Kaiser M-J, De Groote D, Malaise MG. Matrix metalloproteinase-3 serum levels are correlated with disease activity and predict clinical response in rheumatoid arthritis. J Rheumatol 2000; 27:888-93.
Richlin C, Dwyer E, Bucala R, Winchester R. Sustained and distinctive patterns of gene activation in synovial fibroblasts and whole synovial tissue obtained from inflammatory synovitis. Scand J Immunol 1994;40:292-8.
Riisbro R, Christensen IJ, Piironen T, Greenall M, Larsen B, Stephens RW, Han C, Høyer-Hansen G, Smith K, Brünner N, Harris AL. Prognostic significance of soluble urokinase plasminogen activator receptor in serum and cytosol of tumor tissue from patients with primary breast cancer. Clin Cancer Res 2002;8:1132-41.
Rosier RN, O'Keefe RJ. Autocrine regulation of articular cartilage. Instr Course Lect 1998;47:469-75.
Ross JS, Fletcher JA. The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. The Oncologist 1998;3: 237-52.
Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature (Lond.) 1993;362:801-9.
Royston P. Constructing time-specific reference ranges. Statist Med 1991;10: 675-90.
Rønnov-Jessen L, Petersen OW, Bissell MJ. Cellular changes involved in conversion of normal to malignant breast: importance of the stromal reaction. Physiol Rev 1996;76:69-125.
Sacher DB. The problem of postoperative recurrence of Crohn's disease. Med Clin North Am 1990;74:184-8.
Sahai AS, Manocha MS. Chitinases of fungi and plants: their involvement in morphogenesis and host-parasite interaction. FEMS Microbiol Rev 1993;11:317-38.
Saito A, Ozaki K, Fujiwara T, Nakamura Y, Tanigami A. Isolation and maping of a human lung-specific gene, TSA 1902, encoding a novel chitinase family member. Gene 1999;239:325-31.
Sakata M, Masuko-Hongo K, Tsuruha J, Sekine T, Nakamura H, Takigawa M, Nishioka K, Kato T. YKL-39, a human cartilage-related protein, induces arthritis in mice. Clin Exp Rheumatol 2002,20:343-50.
Saleh MN, Goldman SJ, LoBuglio AF, Beall AC, Sabio H, McCord MC, Minasian L, Alpaugh RK, Weiner LM, Munn DH. CD16+ monocytes in patients with cancer: spontaneous elevation and pharmacologic induction by recombinant human macrophage colony-stimulating factor. Blood 1995;85:2910-7.
Sander O, Herborn G, Bock E, Rau R. Prospective six year follow up of patients withdrawn from a randomised study comparing parenteral gold dalt and methotrexate. Ann Rheum Dis 1999;58:281-7.
Sartor RB, Sandborn WJ. Kirsner's inflammatory bowel diseases. Sixth edition. Saunders 2004:105-300.
Saxne T, Heinegard D. Cartilage oligomeric matrix protein: a novel marker of cartilage turnover detectable in synovial fluid and blood. Br J Rheumatol 1992;31:583-91.
Schmidt-Rohlfing B, Gavenis K, Kippels M, Schneider U. New potential markers for cartilage degradation of the knee joint. Scand J Rheumatol 2002;31:151-7.
Schmitt-Graff A, Desmouliere A, Gabbiani G. Heterogeneity of myofibroblast phenotypic features: an example of fibroblastic cell plasticity. Virchows Arch 1994;425:3-24.
Schneider U, Schlegel U, Bauer S, Siebert CH. Molecular markers in the evaluation of autologous chondrocyte implantation. Arthroscopy 2003;19: 397-403.
Schuppan D, Ruehl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. Semin Liver Dis 2001;21:351-72.
Schwartz SM. Smooth muscle migration in atherosclerosis and restenosis. J Clin Invest 1997;99:2814-6.
Scott DL, Grindulis KA, Struthers GR, Coulton BL, Popert AJ, Bacon PA. Progression of radiological changes in rheumatoid arthritis. Ann Rheum Dis 1984;43:8-17.
Scott DL. Prognostic factors in early rheumatoid arthritis. Rheumatology 2000;39(Suppl 1):24-9.
Sekine T, Masuko-Hongo K, Matsui T, Asahara H, Takigawa M, Nishioka K, Kato T. Recognition of YKL-39, a human cartilage related protein, as a target antigen in patients with rheumatoid arthritis. Ann Rheum Dis 2001;60:49-54.
Semino CE, Specht CA, Raimondi A, Robbins PW. Homologs of the Xenopus developmental gene DG42 are present in zebrafish and mouse and are involved in the synthesis of Nod-like chitin oligosaccharides during early embryogenesis. Proc Natl Acad Sci USA 1996;93:4548-53.
Shackel NA, McGuinness PH, Abbott CA, Gorrell MD, McCaughan GW. Novel differential gene expression in human cirrhosis detected by supression subtractive hybridization. Hepatology 2003;38:577-88.
Shackelton LM, Mann DM, Millis AJT. Identification of a 38-kDa heparin-

binding glycoprotein (gp38k) in differentiating vascular smooth muscle cells as a member of a group of proteins associated with tissue remodeling. J Biol Chem 1995;270:13076-83.
Shahabuddin M, Kaslow DC. Plasmodium: Parasite chitinase and its role in malaria transmission. Exp Parasit 1994;79: 85-8.
Sharif M, George E, Shepstone L, Knudson W, Thonar EJ-MA, Cushnaghan J, Dieppe P. Serum hyaluronic acid level as a predictor of disease progression in osteoarthritis of the knee. Arthritis Rheum 1995;38:760-7.
Sharon D, Blackshaw S, Cepko CL, Dryja TP. Profile of the genes expressed in the human peripheral retina, macula, and retinal pigment epithelium determined through serial analysis of gene expression (SAGE). PNAS 2002;99:315-20.
Shostak K, Labunskyy V, Dmitrenko V, Malisheva T, Shamayev M, Rozumenko V, Zozulya Y, Zehetner G, Kavsan V. HC gp-39 gene is upregulated in glioblastoma. Cancer Lett 2003;198:203-10.
Shlopov BV, Lie W-R, Mainardi CL, Cole AA, Chubinskaya S, Hasty KA. Osteoarthritic lesions: involvement of three different collagenases. Arthritis Rheum 1997;40:2065-74.
Shlopov BV, Gumanovskaya ML, Hasty KA. Autocrine regulation of collagenase 3 (matrix metalloproteinase 13) during osteoarthritis. Arthritis Rheum 2000;43:195-205.
Shimizu S, Yamada N, Sawada T, Ikeda K, Kawada N, Seki S, Kanede K, Hirakawa K. In vivo and in vitro interactions between human colon carcinoma cells and hepatic stellate cells. Jpn J Cancer Res 2000;91:1285-95.
Sieper J, Braun J. Pathogenesis of spondylarthropathies: persistent bacterial antigen, autoimmunity, or both? Arthritis Rheum 1995;38:1547-54.
Silver RM, Metcalf JF, Stanley JH, LeRoy EC. Interstitial lung disease in scleroderma. Analysis by bronchoalveolar lavage. Arthritis rheum 1984;27:1254-62.
Sjögren H, Meis-Kindblom JM, Örndal C, Bergh P, Ptaszynski K, Åman P, Kindblom L-G, Stenman G. Studies on the molecular pathogenesis of extraskeletal myxoid chondrosarcoma-cytogenetic, molecular genetic, and cDNA microarray analyses. Am J Pathol 2003;162:781-92.
Smith JA. Neutrophils, host defense, and inflammation: a double-edged sword. J Leukocyte Biol 1994;56:672-86.
Smith MD, Triantafillou S, Parker A, Youssef PP, Coleman M. Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. J Rheumatol 1997;24:365-71.
Soden M, Rooney M, Cullen RA, Whelan A, Feighery C, Bresnihan B. Immunohistological features in the synovium obtained from clinically uninvolved knee joints of patients with rheumatoid arthritis. Br J Rheumatol 1989;28:287-92.
Steck E, Breit S, Breusch SJ, Axt M, Richter W. Enhanced expression of the human chitinase 3-like 2 gene (YKL-39) but not chitinase 3-like 1 gene (YKL-40) in osteoarthritic cartilage. Biochem Biophys Res Commun 2002;299;109-15.
Steenbakkers PGA, Baeten D, Rovers E, Veys EM, Rijnders AWM, Meijerink J, Keyser FD, Boots AMH. Localization of MHC Class II/human cartilage glycoprotein-39 complexes in synovia of rheumatoid arthritis patients using complex-specific monoclonal antibodies. J Immunol 2003;170: 5719-27.
Stokes DG, Liu G, Coimbra IB, Piera-Velazquez S, Crowl RM, Jimenez SA. Assessment of the gene expression profile of differentiated and dedifferentiated human fetal chondrocytes by microarray analysis. Arthritis Rheum 2002;46: 404-19.
Sturgeon C. Practice guidelines for tumor marker use in the clinic. Clin Chem 2002;48:1151-9.
Sun Y-J, Chang N-CA, Hung S-I, Chang AC, Chou C-C, Hsiao CD. The crystal structure of a novel mammalian lectin, Ym1, suggests a saccharide binding site. J Biol Chem 2001;276:17507-14.
Sun X, Gulyas M, Hjerpe A. Mesothelial differentiation as reflected by differential gene expression. Am J Respir Cell Mol Biol 2004;30:510-8.
SundarRaj N, Fite D, Ledbetter S, Chakarvarti S, Hassel JR. Perlecan is a component of cartilage matrix and promotes chondrocyte attachment. J Cell Science 1995;108:2663-72.
Sunderkötter C, Steinbrink K, Goebeler M, Bhardwaj R, Sorg C. Macrophages and angiogenesis. J Leukoc Biol 1994;55: 410-22.
Suzuki T, Hashimoto S, Toyoda N, Nagai S, Yamazaki N, Dong H-Y, Sakai J, Yamashita T, Nukiwa T, Matsushima K. Comprehensive gene expression profile of LPS-stimulated human monocytes by SAGE. Blood 2000;96: 2584-91.
Svendsen AJ, Holm NV, Kyvik K, Petersen PH, Junker P. Relative importance of genetic effects in rheumatoid arthritis: historical cohort study of danish nationwide twin population. BMJ 2001;323:1-5.
Swaak AJ, van Rooyen A, Nieuwenhuis E, Aarden LA. Interleukin-6 (IL-6) in synovial fluid and serum of patients with rheumatoid diseases. Scand J Rheumatol 1988;17: 469-74.
Söletormos G. Serological tumor markers for monitoring breast cancer. Disputats. Lægeforeningens forlag, København 2001.
Taieb J, Mathurin P, Elbin C, Cluzel P, Arce-Vicioso M, Bernard B, Opolon P, Gougerot-Pocidalo MA, Poynard T, Chollet-Martin S. Blood neutrophil functions and cytokine release in severe alcoholic hepatitis: effect of corticosteroids. J Hepatol 2000;32:579-86.
Tak PP, Bresnihan B. The pathogenesis and prevention of joint damage in rheumatoid arthritis. Advances from synovial biopsy and tissue analysis (Review). Arthritis Rheum 2000;43:2619-33.
Takahashi K, Sendai Y, Matsuda Y, Hoshi H, Hiroi M, Araki Y. Mouse oviduct-specific glycoprotein gene: genomic organization and structure of the 5'-flanking regulatory region. Biol Reprod 2000;62:217-26.
Takahashi M, Naito K, Abe M, Sawada T, Nagano A. Relationship between radiographic grading of osteoarthritis and the biochemical markers for arthritis in knee osteoarthritis. Arthritis Res Ther 2004;6:R208-12.
Takehara K. Pathogenesis of systemic sclerosis. J Rheumatol 2003;30:755-9.
Tanwar MK, Gilbert MR, Holland EC. Gene expression microarray analysis reveals YKL-40 to be a potential serum marker for malignant character in human glioma. Cancer Res 2002;62:4364-8.
Tetlow LC, Adlam DJ, Woolley DE. Matrix metalloproteinases and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage. Associations with degenerative changes. Arthritis Rheum 2001;44:585-94.
Thieblemont N, Weiss L, Sadeghi HM, Estcourt C, Haeffner-Cavaillon N. CD14lowCD16high: a cytokine-producing monocyte subset which expands during human immunodeficiency virus infection. Eur J Immunol 1995;25:3418-24.
Thomas CMG, Sweep CGJ. Serum tumor markers: past, state of the art, and future. Int J Biol Markers 2001;16:73-86.
Tran A, Benzaken S, Saint-Paul M-C, Guzman-Granier E, Hastier P, Pradier C, Barjoan EM, Demuth N, Longo F, Rampal P. Chondrex (YKL-40), a potential new serum fibrosis marker in patients with alcoholic liver disease. Eur J Gastroenterol Hepatol 2000;12:989-93.
Vaday GG, Lider O. Extracellular matrix moieties, cytokines, and enzymes: dynamic effects on immune cell behaviour and inflammation. J Leukoc Biol 2000;67:149-59.
Van Aalten DMF, Komander D, Synstad B, Gåseidnes S, Peter MG, Eijsink VGH. Structural insights into the catalytic mechanism of a family 18 exochtinase. PNAS 2001;98: 8979-84.
Van de Loo FAJ, Joosten LAB, van Lent PLEM, Arntz OJ, van den Berg WB. Role of interleukin-1, tumor necrosis factor α, and interleukin-6 in cartilage proteoglycan metabolism and destruction: effect of in situ blocking in murine antigen- and zymosan-induced arthritis. Arthritis Rheum 1995;38:164-72.
Van den Brink HR, van Wijk MJ, Geertzen RG, Bijlsma JW. Influence of corticosteroid pulse therapy on the serum levels of soluble interleukin 2 receptor, interleukin 6 and interleukin 8 in patients with rheumatoid arthritis. J Rheumatol 1994;21:430-4.
Van Gestel AM, Prevoo MLL, van't Hof MA, van Rijswijk MH, van de Putte LBA, van Riel PLCM. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis: comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism criteria. Arthritis Rheum 1996;39:34-40.
Van der Heide A, Remme CA, Hofman DM, Jacobs JWG, Bijlsma JWJ. Prediction of progression of radiologic damage in newly diagnosed rheumatoid arthritis. Arthritis Rheum 1995;38:1466-73.
Van der Heijde DMFM, van't Hof MA, van Riel PLCM, Theunisse LAM, Lubberts EW, van Leeuwen MA et al. Judging disease activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score. Ann Rheum Dis 1990;49:916-20.
Van der Heijde DMFM, van't Hof MA, van Riel PLCM, van Leeuwen MA, van Rijswijk MH, van de Putte LBA. A comparison of validity of single variables and composite indices for measuring disease activity in rheumatoid arthritis. Ann Rheum Dis 1992;51:177-81.
Van der Heijde DMFM. Radiographic imaging: the "gold standard" for assessment of disease progression in rheumatoid arthritis. Rheumatology 2000;39(Suppl 1):9-16.
Van der Horst-Bruinsma I, Speyer I, Visser H, Breedveld FC, Hazes JMW. Diagnosis and course of early-onset arthritis: results of a special early arthritis clinic compared to routine patient care. Br J Rheumatol 1998;37:1084-8.
Van Leeuwen MA, van der Heijde DMFM, van Rijswijk MH, Houtman PM, van Riel PLCM, van de Putte LBA, Limburg PC. Interrelationship of outcome measures and process variables in early rheumatoid arthritis. A comparison of radiological damage, physical disability, joint counts, and acute phase reactants. J Rheumatol 1994;21:425-9.
Van Leeuwen MA, van Rijswijk MH, Sluiter WJ, van Riel PL, Kuper IH, van de Putte LB, Pepys MB, Limburg PC. Individual relationship between progression of radiological damage and the acute phase response in early rheumatoid arthritis. Towards development of a decision support system. J Rheumatol 1997;24:20-7.
Van Meurs J, van Lent P, Holthuysen A, Lambrou D, Bayne E, Singer I, van den Berg W. Active matrix metalloproteinases are present in cartilage during immune complex-mediated arthritis: a pivotal role for stromelysin-1 in cartilage destruction. J Immunol 1999;163:5633-9.

Vanham G, Edmonds K, Qing L, Hom D, Toossi Z, Jones B, Daley CL, Huebner R, Kestens L, Gigase P, Ellner JJ. Generalized immune activation in pulmonary tuberculosis: co-activation with HIV infection. Clin Exp Immunol 1996; 103:30-4.

Varela PF, Llera AS, Mariuzza RA, Tormo J. Crystal structure of imaginal disc growth factor-2. A member of a new family of growth-promoting glycoproteins from Drosophila melanogaster. J Biol Chem 2002;277:13229-36.

Varki A. Does DG42 synthesize hyaluronan or chitin? A controversy about oligosaccharides in vertebrate development. Proc Natl Acad Sci USA 1996;93:4523-5.

Verheiden GFM, Rijnders AWM, Bos E, Coenen-de ROO CJJ, van Staveren CJ, Miltenburg AMM, Meijerink JH, Elewaut D, Keyser F, Veys E, Boots AMH. Human cartilage glycoprotein-39 as a candidate autoantigen in rheumatoid arthritis. Arthritis Rheum 1997;40:1115-25.

Verhoeckx KCM, Bijlsma S, de Groene EM, Witkamp RF, van der Greef J, Rodenburg RJT. A combination of proteomics, principal component analysis and transcriptomics is a powerful tool for the identification of biomakers for macrophage maturation in the U937 cell line. Proteomics 2004;4:1014-28.

Vind I, Johansen JS, Price PA, Munkholm P. Serum YKL-40, a potential new marker of disease activity in patients with inflammatory bowel disease. Scand J Gastroenterol 2003; 38:599-605.

Violin MV, Harlow LA, Woods JM, Campbell PL, Amin MA, Tokuhira M, Koch AE. Treatment with sulfasalazine or sulfapyridine, but not 5-aminosalicylic acid, inhibits basic fibroblast growth factor-induced endothelial cell chemotaxis. Arthritis Rheum 1999;42:1927-35.

Visser H, le Cessie S, Vos K, Breedveld FC, Hazes JMW. How to diagnose rheumatoid arthritis early. A prediction model for persistent (erosive) arthritis. Arthritis Rheum 2002;46: 357-65.

Volck B, Price PA, Johansen JS, Sørensen O, Benfield T, Calafat J, Nielsen HJ, Borregaard N. YKL-40, a mammalian member of the bacterial chitinase family, is a matrix protein of specific granules in human neutrophils. Proc Assoc Am Physicians 1998;110:351-60.

Volck B, Johansen JS, Loew-Friedrich I, Price PA, Oed C, Rosenburg R, Lorenzen I. Changes in serum YKL-40 in patients with rheumatoid arthritis treated with leflunomide Arthritis Rheum 1999a;42(Suppl): abstract 74.

Volck B, Østergaard K, Johansen JS, Garbarsch C, Price PA. The distribution of YKL-40 in osteoarthritic and normal human cartilage. Scand J Rheum 1999b;28:171-9.

Volck B, Johansen JS, Stoltenberg M, Garbarsch C, Price PA, Østergaard M, Østergaard K, Løvgreen-Nielsen P, Sonne-Holm S, Lorenzen I. Studies on YKL-40 in knee joints of patients with rheumatoid arthritis and osteoarthritis. Involvement of YKL-40 in the joint pathology. Osteoarthritis Cartilage 2001;9:203-14.

Vos K, Miltenburg AMM, van Meijgaarden KE, van den Heuvel M, Elferink DG, van Galen PJM, van Hogezand RA, van Vliet-Daskalopoulou E, Ottenhoff THM, Breedveld FC, Boots AMH, de Vries RRP. Cellular immune response to human cartilage glycoprotein-39 (HC gp-39)-derived peptides in rheumatoid arthritis and other inflammatory conditions. Rheumatology 2000a;39:1326-31.

Vos K, Steenbakkers P, Miltenburg AMM, Bos E, van den Heuvel MW, van Hogezand RA, de Vries RRP, Breedveld FC, Boots AMH. Raised human cartilage glycoprotein-39 plasma levels in patients with rheumatoid arthritis and other inflammatory conditions. Ann Rheum Dis 2000b;59: 544-8.

Walsh DA. Angiogenesis and arthritis. Rheumatol 1999;38: 103-12.

Walsh NC, Gravallese EM. Bone loss in inflammatory arthritis: mechanisms and treatment strategies. Curr Opin Rheumatol 2004;16:419-27.

Walter H, Kawashima A, Nebelung W, Neumann W, Roessner A. Immunohistochemical analysis of several proteolytic enzymes as parameters of cartilage degradation. Pathol Res Pract 1998;194:73-81.

Walters MT, Smith JL, Moore K, Evans PR, Cawley MID. An investigation of the action of disease modifying antirheumatic drugs on the rheumatoid synovial membrane: reduction in T lymphocyte subpopulations, and HLA-DP and DQ antigen expression after gold or penicillamine therapy. Ann Rheum Dis 1987;46:7-16.

Ward JM, Yoon M, Anver MR, Haines DC, Kudo G, Gonzalez FJ, Kimura S. Hyalinosis and Ym1/Ym2 gene expression in the stomach and respiratory tract of 129S4/SvJae and wild-type and CYP1A2-null B6,129 mice. Am J Pathol 2001;158: 323-32.

Watanabe T, Kobori K, Miyashita K, Fujii T, Sakai H, Uchida M, Tanaka H. Identification of glutamic acid 204 and aspartic acid 200 in chitinase A1 of Bacillus circulans WL-12 as essential residues for chitinase activity. J Biol Chem 1993;268:18567-72.

Watanabe T, Uchida M, Kobori K, Tanaka H. Site-directed mutagenesis of the Asp-197 and Asp-202 residues in chitinase A1 of bacillus circulans WL-12. Biosci Biotechnol Biochem 1994;58:2283-5.

Weaver AL, Divertis MB, Titus JL. Pulmonary scleroderma. Dis Chest 1968;54:4-12.

Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 1989;320:365-74.

Wells AU, Hansell DM, Rubens MB, Cullinan P, Haslam PL, Black C, Du Bois RM. Fibrosing alveolitis in systemic sclerosis. Bronchoalveolar lavage findings in relation to computed tomographic appearance. Am J Respir Crit Care Med 1994;150:462-8

Werner M, Faser C, Silverberg M. Clinical utility and validation of emerging biochemical markers for mammary adenocarcinoma. Clinical Chemistry 1993;39:2386-96.

Weyand CM, Goronzy JJ. Arterial wall injury in giant cell arteritis. Arthritis Rheum 1999;42:844-53.

Weyand CM. New insights into the pathogenesis of rheumatoid arthritis. Rheumatology 2000a;39(Suppl 1):3-8.

Weyand CM, Fulbright JW, Hunder GG, Evans JM, Goronzy JJ. Treatment of giant cell arteritis: interleukin-6 as a biologic marker of disease activity: Arthritis Rheum 2000b; 43:1041-8.

Weyand CM, Goronzy JJ. Giant-cell arteritis and polymyalgia rheumatica. Ann Intern Med 2003a;139:505-15.

Weyand CM, Goronzy JJ. Medium-and large-vessel vasculitis. N Engl J Med 2003b;349:160-9.

Witt C, Borges AC, John M, Fietze I, Baumann G, Krause A. Pulmonary involvement in diffuse cutaneous systemic sclerosis: broncheoalveolar fluid granulocytosis predicts progression of fibrosing alveolitis. Ann Rheum Dis 1999; 58:635-40.

Wittenhagen P, Kronborg G, Weis N, Nielsen H, Obel N, Pedersen SS, Eugen-Olsen J. The plasma level of soluble urokinase receptor is elevated in patients with Streptococcus pneumoniae bacteraemia and predicts mortality. Clin Microbiol Infect 2004;10:409-15.

Woessner JF, JR, Gunja-Smith Z. Role of metalloproteinases in human osteoarthritis. J Rheumatol 1991;18:99-101.

ten Wolde S, Breedveld FC, Hermans J, Vandenbroucke JP, van de Laar MAFJ, Markusse HM, Janssen M, van den Brink HR, Dijkmans BAC. Randomised placebo-controlled study of stopping second-line drugs in rheumatoid arthritis. Lancet 1996;347:347-52.

Wolfe F, Ross K, Hawley DJ, Roberts FK, Cathey MA. The prognosis of rheumatoid arthritis and undifferentiated polyarthritis syndrome in the clinic: a study of 1141 patients. J Rheumatol 1993;20:2005-9.

Wolfe F, Sharp JT. Radiographic outcome of recent-onset rheumatoid arthritis: a 19-year study of radiographic progression. Arthritis Rheum 1998;41: 1571-82.

Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei X-F, Achong MK. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. J Clin Invest 1998;101:311-20.

Yamada M, Murakami K, Wallingford JC, Yuki Y. Identification of low-abundance proteins of bovine colostral and mature milk using two-dimensional electrophoresis followed by microsequencing and mass spectrometry. Electrophoresis 2002;23:1153-60.

Yamanaka H, Matsuda Y, Tanaka M, Sendo W, Nakajima H, Taniguchi A, Kamatani N. Serum matrix metalloproteinase 3 as a predictor of the degree of joint destruction during the six months after measurement, in patients with early rheumatoid arthritis. Arthritis Rheum 2000;43:852-8.

Yoshihara Y, Obata K, Fujimoto N, Yamashita K, Hayakawa T, Shimmei M. Increased levels of stromelysin-1 and tissue inhibitor of metalloproteinases-1 in sera from patients with rheumatoid arthritis. Arthritis Rheum 1995;38:969-75.

Young A, van der Heijde DM. Can we predict aggressive disease? Baillieres Clin Rheumatol 1997;11:27-48.

Young RD, Lawrence PA, Duance VC, Aigner T, Monaghan P. Immunolocalization of collagen types II and III in single fibrils of human articular cartilage. J Histochem Cytochem 2000;48:423-32.

Youssef PP, Cormack J, Evill CA, Peter DT, Roberts-Thomson PJ, Ahern MJ,Smith MD. Neutrophil trafficking into inflamed joints in patients with rheumatoid arthritis, and the effects of methylprednisolone. Arthritis Rheum 1996; 39:216-25.

Youssef PP, Haynes DR, Triantafillou S, Parker A, Gamble JR, Roberts-Thomson PJ, Ahern MJ, Smith MD. Effects of pulse methylprednisolone on inflammatory mediators in peripheral blood, synovial fluid, and synovial membrane in rheumatoid arthritis. Arthritis Rheum 1997;40: 1400-8.

Ziegler-Heitbrock HWL. Heterogeneity of human blood monocytes: the CD14+,CD16+ subpopulation. Immunol Today 1996;17:424-8.

Zvaifler NJ, Firestein GS. Pannus and pannocytes. Alternative models of joint destruction in rheumatoid arthritis. Arthritis Rheum 1994;37:783-9.

Østergaard C, Johansen JS, Benfield T, Price PA, Lundgren JD. YKL-40 is elevated in cerebrospinal fluid from patients with purulent meningitis. Clin Diagn Lab Immun 2002;9: 598-604.