Gc-globulin in liver disease

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BACKGROUND

GC-GLOBULIN

The α-2-globulin Gc-globulin (other names: group-specific component, vitamin D-binding protein) is a multifunctional protein [8-10], and its main physiological importance is probably binding of actin. Gc-globulin scavenges monomeric actin and together with gelsolin constitutes the extracellular actin scavenger system [11].

Gc-globulin was first described in 1959 [12]. It is encoded for on the long arm of chromosome 4 (4q11-13). The Gc-globulin gene has been identified [13]: the protein consists of 458 amino acid residues (and a 16 amino acid tail) and contains 14 disulfide bridges [9;14]. The molecular weight is approximately 56,000 Daltons, but the exact weight depends on the amount of glycosylation.

The serum concentration of Gc-globulin in normal individuals is approximately 300 to 500 mg/L (5.4 to 8.9 x 10^{-6}M), thus constituting ca. 6 per cent of alpha-globulins [8]. Serum Gc-globulin concentration is stable from full-term birth throughout life and is not related to age or gender [15-17]. It varies slightly with the phenotype since people with phenotype Gc1-1 have higher concentrations than people with phenotype Gc2-1 that again have higher Gc-globulin concentrations than Gc2-2 [15;18-20]. Also, infants younger than 6 months of age have lower Gc-globulin levels in the summer than in the winter, whereas there is no seasonal variation in adults [21]. There seems to be a diurnal rhytm with lowest levels observed at night [22]. Gc-globulin levels may be altered in pathologic and physiologic conditions, either with decreased levels (e.g., in liver disease or in sepsis) or with increased levels (e.g., in pregnancy).

Phylogenetically, Gc-globulin is believed to be an old protein, approximately 600 million years old [23]. It has been well conserved throughout evolution and it is present in all vertebralian animals studied. Five hundred million years ago, the ancestral Gc-globulin gene split up, and Gc-globulin, albumin, alpha-fetoprotein, alpha-albumin, and afamin developed, constituting the so-called *albumin gene superfamily* [23-28]. No homozygote null Gc-globulin allele has been detected in humans despite testing of many thousand serum samples [29]. This could indicate that presence of Gc-globulin is vital for the organism and that null Gc-globulin mutations might be lethal [11]. However, this view has been been challenged by a mouse knockout model (Gc-globulin -/-) that showed normal viability and fertility of the animals [30].

Gc-globulin is a 3-domain protein [31]; domain I binds to vitamin D metabolites whereas domain III contains the actin-binding site with a very good structural fit to a large groove between actin subdomain 1 and 3 [32]. The intermolecular contact between the 2 proteins is large (3400-3600 Å^2) [32;33].

Gc-globulin kinetics has not been studied in great detail. Gc-globulin is synthesized almost solely in the liver [8], although minimal Gc-globulin mRNA expression (in the rat) has also been documented in other tissues, including the kidney, yolc sac, and testis suggesting a minimal extrahepatic synthesis [26]. In healthy human volunteers the exchangeable pool of Gc-globulin was evaluated to be 3 gram [34]. The half-life of uncomplexed Gc-globulin is approximately 48 hours in man [34], 17 hours in the rabbit [35], and 10 hours in the rat [36]. Actin-complexed Gc-globulin has a much shorter half-life, ~ 60 minutes in the rabbit [35] and 30 minutes in the rat [36].

ACTIN

Actin is a major cellular protein component constituting up to 20 per cent of the cell protein content and acting as cytoskeleton for hepatocytes and most other cells [37;38]. Actin filament rearrangement enables certain cells to form pseudopods and allow »cell crawling« [39;40], and actin and myosin filaments constitute the major part of the contractile system in skeletal muscle [37;41]. Monomeric actin is globular in form (G-actin) and the polymeric form of actin is caused by assembly of many G-actin units to form linear filaments (F-actin), up to 10 μm in length [42]. Under physiologic conditions, such as in plasma or isotonic saline, G-actin will spontaneously form F-actin.

Actin in the circulation may influence hemostasis in several ways: by inducing platelet aggregation [43;44], by acting as a plasmin inhibitor (45), or by interacting with the fibrinolytic system [46]. Further, incorporation of actin filaments into fibrin clots changes clot rheology by inhibiting strain-hardening, making the clot more brittle than without incorporated actin [47;48]. Overall, intravascular actin in plasma influences the organism towards increased thrombosis and decreased fibrinolysis.

THE EXTRACELLULAR ACTIN SCAVENGER SYSTEM

Disruption of the integrity of the cell membrane will cause release of actin to the extracellular space. This disruption may be part of normal cell turnover or may stem from cellular necrosis caused by pathologic conditions leading to cell necrosis [11]. Release of actin from damaged or necrotic cells into the circulation may have severe side effects for the organism [49]. In a very important study, *Haddad et al* demontrated that infusion of high doses of G-actin in the rabbit caused rapid and fatal formation of massive actin filament-containing thrombi in arterioles and capillaries of pulmonary veins and there was also evidence of endothelial injury [49]. Thrombi formation was not observed when the same amount of G-actin was preincubated with Gc-globulin. Further evidence for the deleterious effects of actin was demonstrated by *Erukhimov et al* showing that actin from necrotic cells could produce a direct injury to pulmonary endothelial cells [50].

These and other observations lead to the suggestion of the presence in plasma of an extracellular actin scavenger system, consisting of two plasma proteins: Gc-globulin and gelsolin [11;35;51;52]. Gelsolin, synthesized in skeletal muscle [53], depolymerizes *polymeric actin*, F-actin, by capping [54], annealing, and severing [42;55] the protein at a 1:2 molar ratio [56], whereas Gc-globulin binds with high affinity (Kd= 10^{-9} M) to *monomeric actin* at a 1:1 molar ratio, thus forming a Gc-globulin:actin complex [57]. The complex is cleared by parenchymal and endothelial cells [58] or Kupffer cells in the liver [59] (Figure 1).

OTHER FUNCTIONS OF GC-GLOBULIN

Gc-globulin is a multifunctional protein (31). Its presumed physiologic functions are listed in Table 1.

Vitamin D-binding: Binding of vitamin D metabolites (primarily 25-OH vitamin D and 1,25-di-OH vitamin D) was the first described function of Gc-globulin, hence, the synonym vitamin D-binding protein (VDBP or DBP) [60;61]. Gc-globulin's sterol-binding (vitamin D-binding) site is located at the amino-terminal end of the protein (domain I), as opposed to the actin-binding site at the

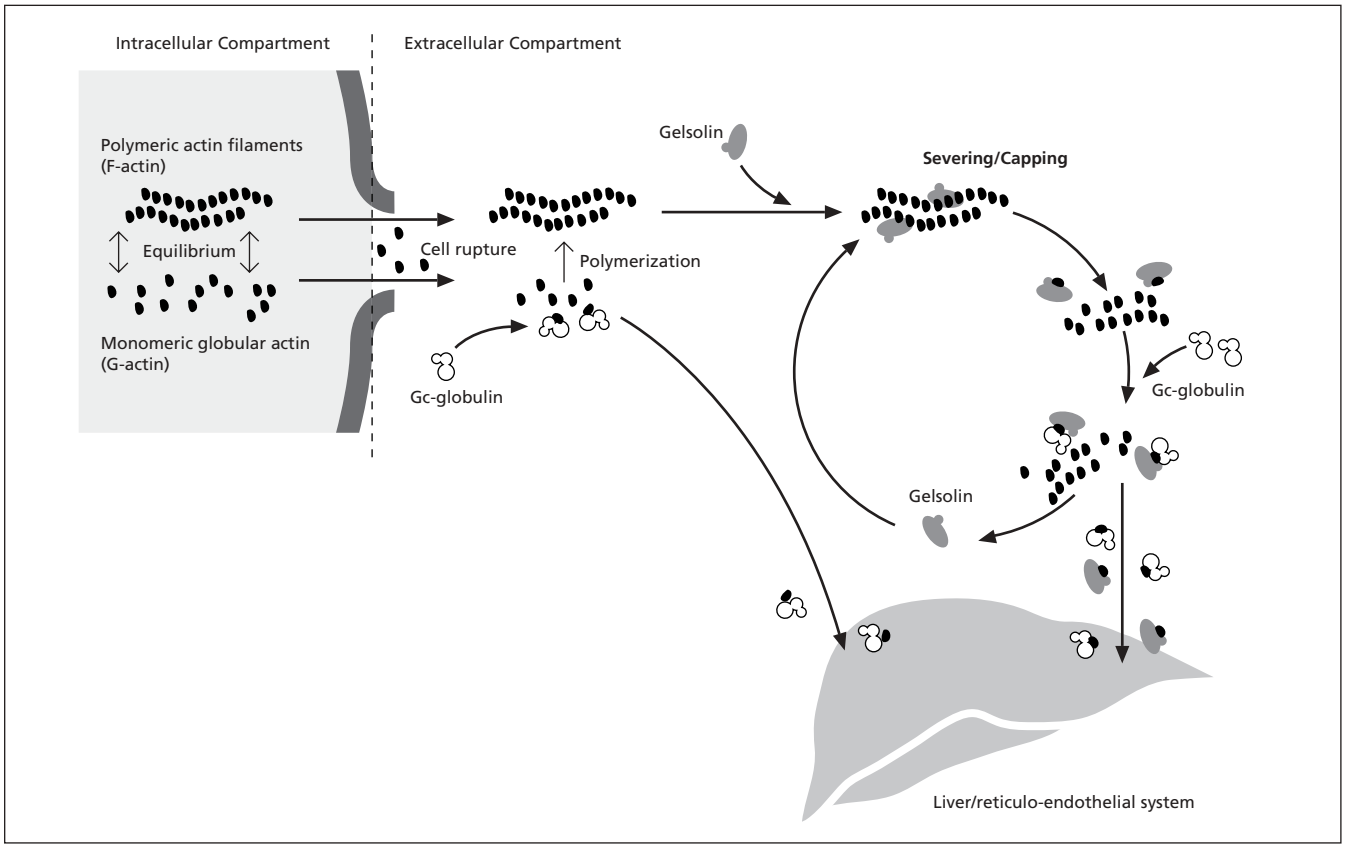

Figure 1. The presumed action of the extracellular actin scavenger system. Upon cell necrosis actin is released from the cell to the extracellular space in both polymeric and monomeric form. Gelsolin depolymerizes polymeric (filamentous) actin by capping, severing and annealing to form monomeric (globular) actin, which is next complexed to Gc-globulin. This complex is presumably taken up in hepatic Kupffer cells/the reticulo-endothelial system. (Reprinted, with permission from K Bangert, Antibodyshop).

carboxy-terminal end (domain III) [62]. Binding of sterols does not affect the actin-binding property or capacity [62]. Vitamin D-binding occupies less than 5% of the normal sterol-binding capacity [8], and Gc-globulin levels do not correlate with levels of vitamin D metabolites [63].

Precursor for macrophage activating factor: In 1991, *Yamamoto et al* described for the first time the role of Gc-globulin as a precursor of macrophage activating factor (Gc-MAF). In a series of experiments [64-66], this group described the conversion of Gc-globulin to MAF; Gc-globulin is modified by the combined action of membrane-bound β-galactosidase of B-lymphocytes and sialidase of T-lymphocytes to form Gc-MAF [64]. Gc-MAF acts as a switch to turn on macrophage activity at sites of infection and inflammation [67] and may cause apoptosis of these macrophages when they are no longer needed [68]. Some AIDS and cancer patients produce α-N-acetylgalactosaminidase, an enzyme that deglycosylates Gc-globulin, inhibiting the formation of Gc-MAF [69-71], and possibly contributing to the immunosuppression observed in these patients.

Also, Gc-MAF has direct antiangiogenic effects on endothelial cells [72;73] and an overexpression of Gc-globulin has been observed in tumor-bearing breasts [74].

Co-chemotactic effect for C5a and C5a des Arg: Gc-globulin enhances the neutrophil chemotactic effect of C5a and C5a des Arg for neutrophils and macrophages [75-78]. The mechanism seems to be regulated by elastase from neutrophils [79] and is related to a direct binding to C5a des Arg, since the Gc-globulin:C5a des Arg complex increases the number of C5a des Arg molecules/unit on the polymorphonuclear leucocytes [80]. Gc-globulin's C5a chemotactic cofactor function is mediated by CD44 and annexin A2, both involved in cell movement [81;82].

Natural killer cell enhancement: Anti-Gc-globulin antiserum inhibits the activity of natural killer (NK) cells on peripheral blood lymphocytes *in vitro* [83;84]. This inhibitory effect is blocked by addition of purified Gc-globulin. Thus, Gc-globulin seems to be associated with NK cytolysis in the post-binding cytolytic phase.

Binding of arachidonic acid and endotoxin: Under normal circumstances, 75-80% of serum arachidonic acid, parent molecule for the cyclooxygenase pathway, is bound to Gc-globulin [85-87]. Addition of Gc-globulin to protein preparations leads to a 40% decreased endotoxin neutralizing activity [88].

Others: Surface-bound Gc-globulin has been observed in a number of cells, including monocytes [89], B-lymphocytes [90;91]), human placental trophoblasts, and neutrophils [92]. The Gc-globulin is probably plasma-derived [93], and the physiologic importance may be related to cell differentiation [94]; however, this issue is not yet clarified. Other functions include the recent finding, that Gc-MAF can stimulate osteoclast activity and bone resorption in an extracellular calcium-dependent way [95].

In conclusion, Gc-globulin is a multifunctional protein, mainly associated with the nonspecific innate immune system, vitamin D-binding, and actin-scavenging. The priority of these functions is not yet clear. Further, to what extent each function influences the other functions remains to be studied.

ANALYSIS OF GC-GLOBULIN

The following Gc-globulin definitions are useful when reading this thesis:

Total Gc-globulin: the total concentration of Gc-globulin in serum.

Complex ratio: the percentage of total Gc-globulin complexed to actin.

Free Gc-globulin: the concentration of Gc-globulin not complexed to actin. Can be calculated as total Gc-globulin x (1 – complex ratio).

Bound Gc-globulin: the concentration of Gc-globulin complexed to actin. Can be calculated as total Gc-globulin x complex ratio.

Several methods have been developed to measure serum Gc-globulin concentrations. In normal individuals, the mean and range for

Table 1. Gc-globulin functions

Actin-scavenging
Vitamin D-binding
Endotoxin binding
Co-chemotactic effect for C5a
Natural killer cell activation
Precursor for macrophage activating factor
Others

Table 2. Methods for determining serum Gc-globulin and complex ratio. Normal levels. Data are given as mean or median ± SD (range).

Method	Ref.	Gc-globulin (mg/L)	Complex ratio (%)
Single radial immunodiffusion	(97)	422 (315-523)	N/A
	(101)	522 ± 62 (460–584)	N/A
	(63)	340 ± 61	N/A
	(102)	294 ± 3	N/A
	(20)	292 ± 33	N/A
Rocket immuno-electrophoresis	(96)	357 ± 132 (273–529)	32 ± 8 (20–44)
	(103)	393 ± 65	N/A
	(104)	404 ± 124	N/A
	(1)	342 ± 61 (240-482)	13 ± 13 (0-27)
	(2)	365 ± 57 (265-390)	11 ± 12 (2-28)
	(4)	340 ± 35 (265-390)	13 ± 8 (2-28)
Radioimmunoassay	(105)	347 ± 5	N/A
Western blot	(106)	288 (138-427)	N/A
Nephelometry	(106)	292 (163–509)	N/A
	(107)	394 (320–460)	N/A
	(7)	(350-500)	N/A
ELISA	(109)	355 ± 99 (220–606)	N/A
	(100)	305 (176-623)	N/A
Turbidimetry	(108)	256 ± 24	N/A

N/A = not available.

serum Gc-globulin did not differ very much among the methods (Table 2). So far, only rocket immunoelectrophoresis [1;2;4;96], non-SDS Western blot with polyacrylamide gel electrophoresis (PAGE) [97-99], and crossed immunoelectrophoresis [100] allow for calculation of the actin complex ratio. Single radial immunodiffusion [20;63;97;101;102], rocket immunoelectrophoresis [1;96;103; 104], radioimmunoassays [105], and Western blot [106] are rather slow and cumbersome techniques, mostly suited for scientific projects. Furthermore, the accuracy of these techniques is not impressive. For details regarding rocket immunoelectrophoresis, see Appendix. In recent years, newer techniques including nephelometry [7;106;107], turbidimetry [108], and ELISA [100;109] have been developed. These methods are more accurate and are also much faster than »first generation techniques«. Thus, they could be more useful in a clinical setting. A new automated commercial ELISA method to determine free Gc-globulin is currently under evaluation [110;111].

Influence of fresh frozen plasma infusions on Gc-globulin values: fresh frozen plasma (FFP) contains plasma proteins in normal concentrations, and FFP infusions thus iatrogenically increase serum Gc-globulin concentrations in patients with reduced levels. We studied the duration of increased Gc-globulin values; pre-infusion values resumed 4 - 6 hours after infusion of one to two units of FFP [1]. High volume plasmapheresis, used in the treatment of severe liver dysfunction [112;113], involving a plasma exchange of 8-12 liters of plasma (15% of bodyweight) caused increased serum Gc-globulin values that lasted for approximately 24 hours [5;6].

ACETAMINOPHEN (PARACETAMOL) OVERDOSE

Acetaminophen is a very safe drug when ingestion does not exceed the daily recommended maximum of 4 gram/day. However, acetaminophen is also a dose-dependent hepatotoxin and its therapeutic index is very low. The typical pathological finding is centrilobular (zone III) necrosis where up to 90 per cent of hepatocytes may be necrotic [114-116]. Aminotransferase levels are usually very high [114]. Hepatotoxicity is more likely to occur if the antidote N-acetylcysteine (NAC) is instituted late after ingestion or if the patient is a chronic alcoholic [117;118].

Development of hepatic encephalopathy (and therefore, by definition, acute liver failure (ALF)) is also related to delay to NAC treatment [119] and is most likely to occur in patients with accidental overdose [120]. Acetaminophen overdose is the commonest cause of ALF in Denmark [121], the United Kingdom [122], and now also in the United States [123].

Experimental studies on acetaminophen-induced ALF have shown the actin-scavenger system to be stressed, as evaluated by decreased Gc-globulin levels. Also, a very high proportion of Gc-globulin:actin complexes (i.e., a high complex ratio) was observed in animals with severe liver damage [98;99].

So far, 5 clinical studies on Gc-globulin in acetaminophen overdose have been published (Table 3). Patients with ALF have been in-

Table 3. Gc-globulin in acetaminophen overdose, clinical studies.

	Location	N	Analysis method	Observation period	Characteristics and findings		
Lee et al (97)	London	47*	PAGE Western blot	Daily samples, unclear period	All had HE. Admission Gc was 55 ± 13 mg/L in nonsurvivors and 82 ± 11 mg/L in survivors. Complex ratio was measured but not reported. Free Gc calculated		
Schiødt et al (1)	Copen-hagen	18	RIEP	Every 3 hours, up to 30 hours after admission	No HE (n=10)	HE (n=8)	
					Admission Gc: 245 ± 82 mg/L Nadir Gc: 197 ± 72 mg/L Admission complex ratio: 16 ± 23% Peak complex ratio: 49 ± 13%	Admission Gc: 107 ± 39 mg/L Nadir Gc: 84 ± 18 mg/L Admission complex ratio: 29 ±% Peak complex ratio: 70 ± 14%	
Schiødt et al (2)	Copen-hagen	18	RIEP	Admission	All had HE. Gc: 174 ± 91 mg/L Complex ratio: 46 ± 31%		
Schiødt et al (6)	Copen-hagen	84	RIEP	Twice daily, entire hospital stay	Non-HEPTOX (n=32)	HEPTOX (n=37)	HE (n=15)
					Nadir Gc: 310 ± 90 mg/L Nadir free Gc: 262 ± 103 mg/L Peak complex ratio: 18 ± 20%	Nadir Gc: 148 ± 67 mg/L Nadir free Gc: 86 ± 56 mg/L Peak complex ratio: 47 ± 18%	Nadir Gc: 97 ± 50 mg/L Nadir free Gc: 50 ± 32 mg/L Peak complex ratio: 57 ± 18%
Schiødt et al (7)	U.S.A. (multi-center)	76	Nephe-lometry	Day 1 and 2	All had HE. Gc: 114 (range 34-307) mg/L		

HE = hepatic encephalopathy. HEPTOX = ALT over 1,000 U/L but no HE. Gc = total Gc-globulin. Non-HEPTOX = ALT below 1,000 U/L and no HE. PAGE = polyacrylamide electrophoresis. RIEP = rocket immunoelectrophoresis. *) 39 of these patients had acetaminophen-induced ALF.

cluded in all these studies. Only 2 studies have reported Gc-globulin levels in patients without ALF [1;6]. These 2 studies have also described the temporal profile of serum Gc-globulin in acetaminophen overdose. In study [1], patients were followed for approximately 24 hours after inclusion in the study. Levels were lower among patients with hepatic encephalopathy than in those without. Gc-globulin levels did not change much over that period of time (Figure 2). In study [6], patients were followed over the entire hospital stay and several important observations could be made. Patients were divided into 3 groups according to degree of liver injury: 1) no or minimal injury, 2) moderate injury with high aminotransferase levels but no ALF, and finally 3) patients with ALF. Total and free Gc-globulin concentrations and complex ratio levels were unaffected in group 1 (Figure 3 and Figure 4), in contrast to the decrease in plasma coagulation factor II, VII, X activities (Figure 5) probably mediated by the anti-coagulant effect of the antidote NAC [124;125]. In contrast, patients in group 2 displayed signs of affection of the actin scavenger system, since total and free Gc-globulin levels fell to less than half of normal values, with nadir values occurring ca. 3 days after acetaminophen ingestion at the same time where complex ratio and aminotransferese levels peaked (Figures 3-5). These changes were more accentuated in group 3 where nadir and peak levels were even more abnormal. For groups 2 and 3 levels gradually normalized hereafter even though they were not yet in the normal range 7 days into overdose.

In general, total Gc-globulin levels are decreased in all patients with hepatotoxicity (Table 3). Patients with ALF have total Gc-globulin concentrations of approximately 100 mg/L which is less than one third of normal values.

Bound Gc-globulin remained normal at all times for all 3 groups. If one excludes the possibility of a methodology bias (the fact that bound Gc-globulin is not measured directly but is a product of 2 measured variables) then this could indicate that bound Gc-globulin concentration is narrowly regulated. I speculate that bound Gc-globulin levels may regulate Gc-globulin:actin complex metabolism even in the failing liver. However, the exact mechanism of the uptake of Gc-globulin:actin complexes in the liver is largely unknown so further studies should elucidate this.

To summarize, levels of Gc-globulin and actin complex ratio are affected in patients with acetaminophen overdose if hepatotoxicity is present, and patients with ALF have the most depressed serum levels. The time profile shows a close correlation between peak complex ratio and peak aminotransferase levels indicating that actin released from the necrotic hepatocytes contribute to the stress on the actin scavenger system in acetaminophen overdose.

ACUTE LIVER FAILURE (FULMINANT HEPATIC FAILURE) AND PROGNOSIS

It is safe to say that acute liver failure (ALF) is one of the most dramatic conditions in medicine. The failing liver leads by definition to hepatic encephalopathy within a short time after initial symptoms [126] and may also lead to a cascade of organ failures including renal failure, circulatory collapse, and pulmonary dysfunction. Further, severe infections, deep coagulopathy, and the risk of cerebral edema, intracranial hypertension, and cerebral herniation adds to the picture of an extreme disease entity. Not surprisingly, the mortality rate in ALF has historically been very high, with survival being the exception to the rule [127-130]. The increased use of intensive

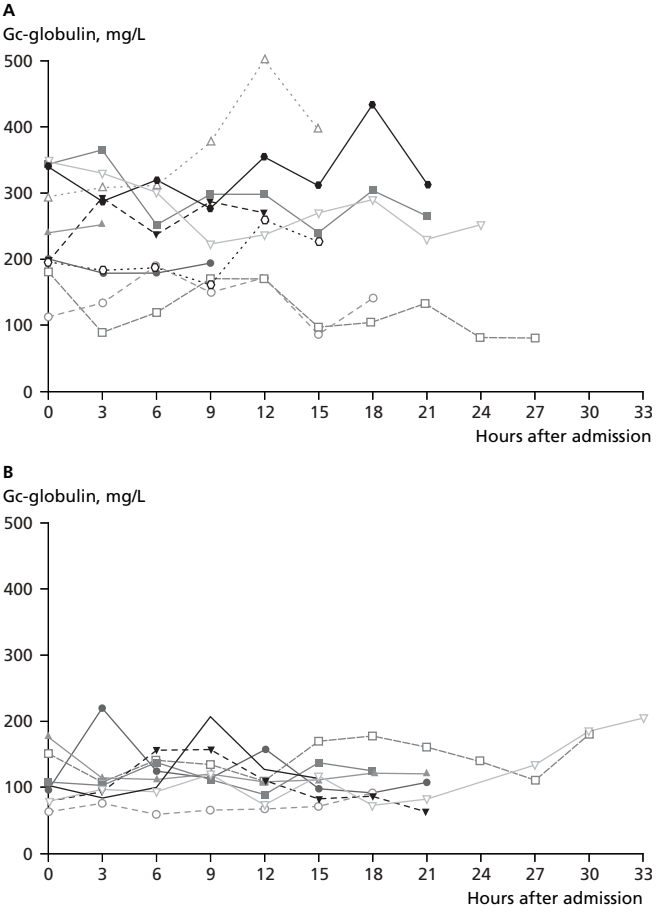

Figure 2. Serum Gc-globulin in 18 patients with acetaminophen (paracetamol) overdose. **A**: ten patients without hepatic encephalopathy (HE). **B**: eight patients with HE.

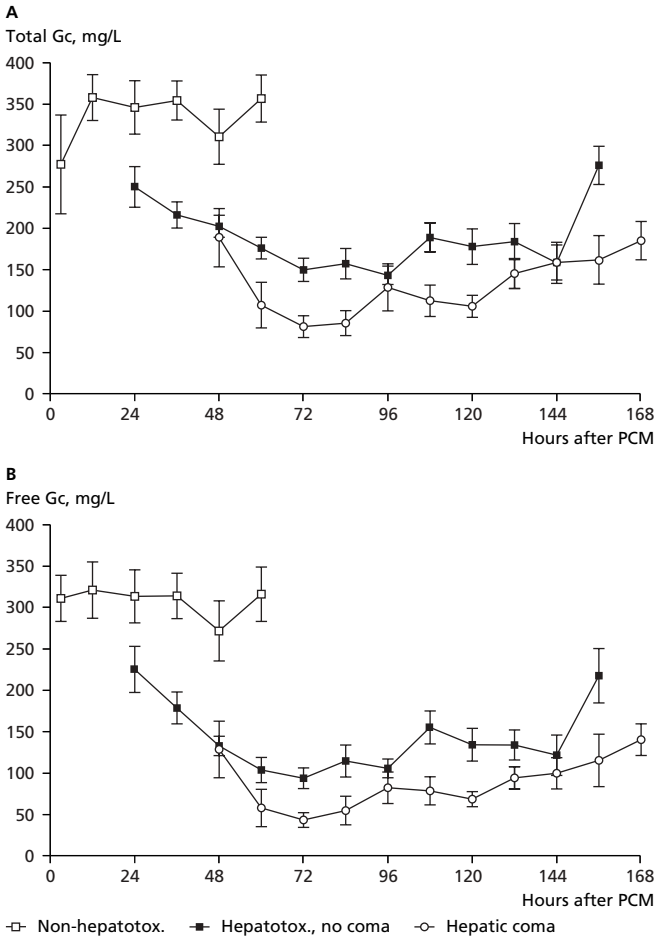

Figure 3. The temporal profile of total and free Gc-globulin in paracetamol overdose. Three grops were identified: 1) Patients without elevation of transaminases (non-hepatotox, filled circles), 2) Patients with transaminases > 1,000 U/L but no hepatic encephalopathy (hepatotox, no coma, open squares), and 3) Patients with acute liver failure (hepatic coma, filled squares). Time denotes hours after intake of paracetamol (PCM).

care monitoring and therapy and the advent of liver transplantation as a treatment option has improved prognosis considerably; however, mortality rates still remain at approximately 40 to 50%, even in the most experienced centers [123;131;132]. Favorable etiologies include acetaminophen, hepatitis A, pregnancy, and shock liver where the spontaneous survival rate (i.e., survival without liver transplantation) is greater than 50% as compared to a lower than 25% spontaneous survival rate for all other etiologies [123;133;134].

Serum Gc-globulin in ALF was first studied *by Lee & Galbraith's* group. Their initial reports, including relatively few patients, documented dramatic changes in Gc-globulin levels and complex ratio values [96;101;109], suggesting a severe reduction of Gc-globulin's actin-scavenging capacity. These studies were supported by similar findings in animal models of acetaminophen-induced ALF where, typically, total Gc-globulin levels were low and actin complex ratios were high and the time of the extremes correlated with the peak of aminotransferase levels [98;99].

So far, eleven clinical studies regarding Gc-globulin and ALF have been published (Table 4). Eight studies [1;2;6;7;96;101;106;109] have reported on total Gc-globulin levels. The results were very similar among the studies; Gc-globulin concentrations were reduced to between 25% and 49% of normal (Table 4). Free Gc-globulin levels (reported in 5 studies) were even lower, between 12% and 26% of normal, and complex ratios (reported in 5 studies) were elevated in all papers. Thus, the stress on the actin scavenger system in ALF seems very obvious.

Since the hepatic necrosis is so overwhelming in acetaminophen hepatotoxicity one would *a priori* assume Gc-globulin levels in this group to be lower than in the nonacetaminophen group. In fact, the opposite is true, since patients with acetaminophen-induced ALF have higher Gc-globulin concentrations than those patients with nonacetaminophen-induced ALF [2;7]. This could be due to a better prognosis in acetaminophen-induced ALF where the spontaneous survival chances are greater than 50% compared to a lower than 25% chance in nonacetaminophen-induced ALF [123;134]. In fact, spontaneous survivors of acetaminophen etiology had the same Gc-globulin levels as survivors of nonacetaminophen etiology in one study [7] – whereas there was a significant difference in Gc-globulin levels among nonsurvivors of the 2 groups. Another explanation for the increased Gc-globulin levels in acetaminophen-induced ALF may be that this disease is a »single-hit« disease where acetaminophen – or rather its highly reactive metabolite NAPQI – causes severe hepatocellular damage [135;136]. However, further damage is stopped once antidote treatment is instituted, in contrast to the continuous damage inflicted to the liver by for instance acute viral hepatitis B or by acute Wilson's disease.

Several prognostic models are available to determine outcome in ALF, including the British King's College Hospital criteria [137], the French Clichy criteria [138], plasma coagulations factors [139], arterial lactate [140], arterial ammonia [141], phosphate [142], and serum levels of alpha-fetoprotein [143;144]. The King's College Hospital criteria are still the most commonly used, despite being almost 20 years old [137]. However, the predictive accuracy of a model seems to decrease when applied on patients from other regions or countries than where they originated [145-147].

Three studies have reported on Gc-globulin and prognosis in ALF (Table 5), and the final results of an ongoing study from the USA are pending [148]. Preceeding these studies, one paper reported the

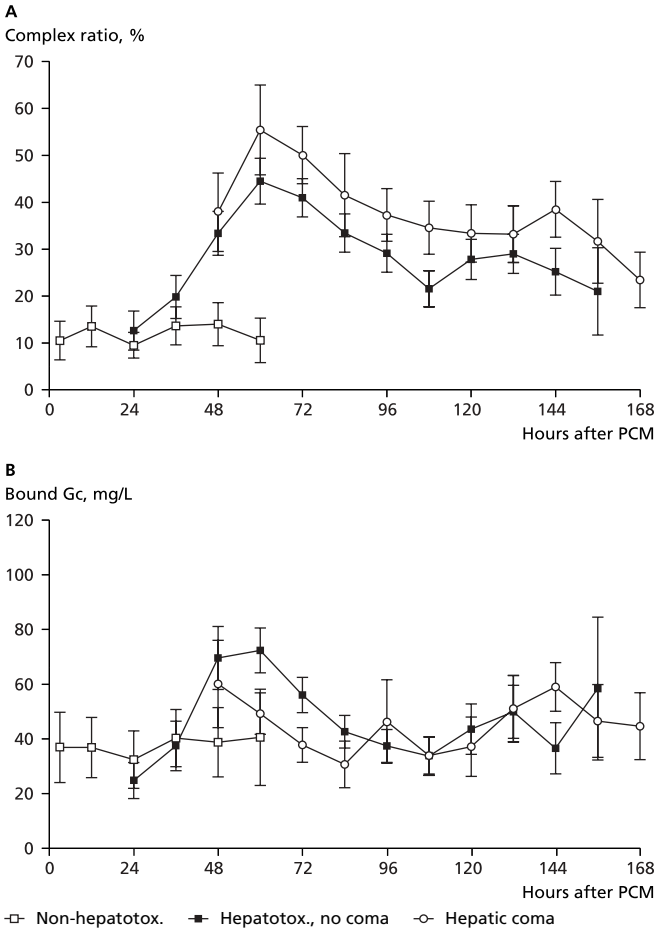

Figure 4. The temporal profile of complex ratio and bound Gc-globulin in paracetamol overdose. The same 3 groups as in Figure 3. Time denotes hours after intake of paracetamol (PCM).

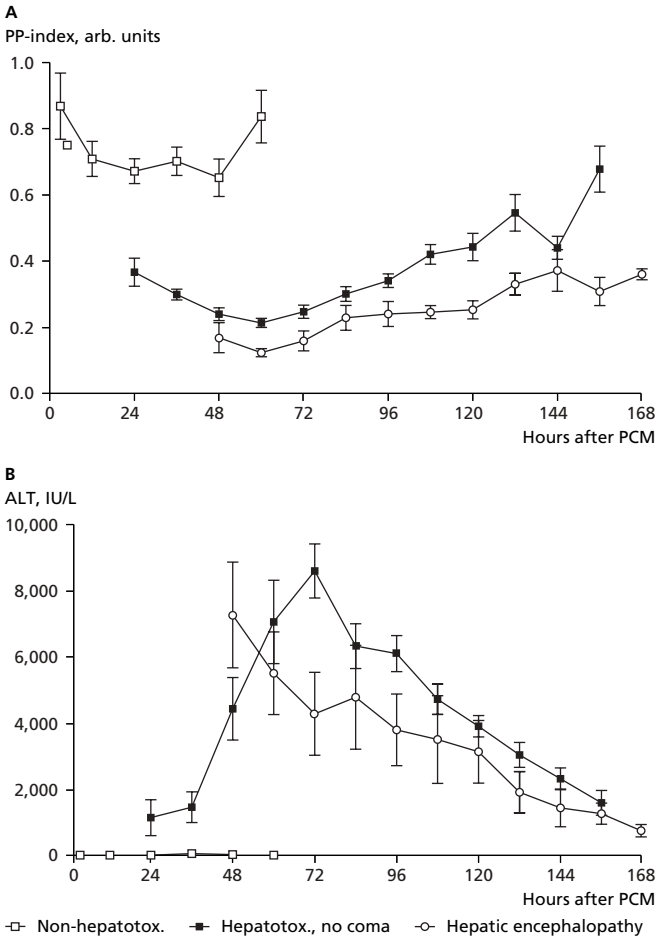

Figure 5. The temporal profile of PP-index (activity of plasma coagulation factors II, VII, and X) and alanine aminotransferase (ALT) in paracetamol overdose. The same 3 groups as in Figures 3 and 4. Time denotes hours after intake of paracetamol (PCM).

value of actin complex ratio in seven patients with ALF [101], and found that nonsurvivors had higher complex ratio than survivors. Two studies [2;7] described the prognostic value of total Gc-globulin concentrations. In general, the prognostic value was better for nonacetaminophen patients. The prognostic cutoff levels for total Gc-globulin were quite similar in the 2 studies, 100 mg/L and 80 mg/L, respectively. In fact, in one study [7], there was no difference in total Gc-globulin levels between survivors and nonsurvivors of acetaminophen-induced ALF. For the nonacetaminophen groups, the positive prognostic values were 79% and 85%, respectively, whereas the negative predictive values were lower, 60% and 43%, respectively (Table 5). One study [97] reported on the prognostic value of free Gc-globulin and here the test seems to yield prognostic information in acetaminophen patients also. The prognostic cutoff level for free Gc-globulin was 34 mg/L and day 2 data seemed to give better prognostic information than admission levels. In the above mentioned abstract [148] the prognostic cutoff level was 40 mg/L and thus very close to that reported in *Lee et al's* study. Even though the predictive accuracy of Gc-globulin was rather low, it was in the same range as that of the King's College Hospital criteria in all 3 studies, demonstrating the imprecision of all prognostic markers.

The ideal prognostic marker is 100% accurate, with a perfect discrimination between positives and negatives. Unfortunately, no such marker exists. With the advent of acute liver transplantation as a treatment option it is even more important to have accurate prognostic markers, since we don't want to transplant those patients who would survive spontaneously. Conversely, we want to make an early request for a liver donor in patients with a low likelihood of survival. Most studies who described prognosis in ALF give data on sensitivity and specificity. A meta-analysis showed the sensitivity to be lower than the specificity in most studies [149] meaning that it is apparently easier to identify survivors than nonsurvivors. For the clinician, however, these data are less useful since they are *post festum*. In that respect, positive and negative predictive values are more important.

Liver transplantation has not made is easier to develop diagnostic tests since the true (untransplanted) outcome is never known in patients who are transplanted. Of course, these patients could be excluded from analyses. On the other hand, almost one quarter of the ALF patients are transplanted [123] so an unfair bias would be introduced in the analysis if these patients were excluded. Most studies have opted to include transplanted patients and consider them together with nonsurvivors [7;150], in contrast to the 'spontaneous survivors' who do not undergo transplantation.

The prognosis of ALF has improved over time [151] and the studies listed in table 5 span over 4 decades, from the 1970's and 1980's [2;97] to the 2000's [7]. Therefore, it is interesting that the predictive values (and sensitivity and specificity) are so relatively unchanged over the years. The robustness of such prognostic tests could also explain why the King's College Hospital criteria are still so widely used, even though an attempt to improve the criteria with the inclusion of arterial lactate has recently been suggested [140]. ALF is such a complex disease, so it is not unexpected that a single prognostic test cannot be perfectly accurate. Therefore, in the future, total and free Gc-globulin levels should be tested with prognostic markers that display other aspects of liver function, e.g. liver regeneration (alpha-feto protein), hepatocyte necrosis (ferritin), or features of infections (SIRS).

MULTIPLE ORGAN FAILURE IN ACUTE LIVER FAILURE

A failing liver is the initial event in ALF that, by definition, leads to hepatic encephalopathy. However, other organs may also fail contributing to the morbidity and mortality and making ALF such a challenging condition [152-154]. Sepsis or evidence of the systemic inflammatory response syndrome (SIRS) are probably of paramount importance for the development of multiple organ failure (MOF) in ALF [155] and may also lead to worsening of hepatic encephalopathy [156]. Patients with ALF often develop renal failure, arterial hypotension, severe infections, and occasionally pulmonary dysfunction [151]. However, the most feared complication in ALF is the development of cerebral edema and intracranial hypertension where cerebral incarceration is imminent [157;158]. Multiple organ dysfunction (MOD) is perhaps a better term than MOF since MOD describes a continuum of dysfunction whereas MOF is a dichotomous evaluation with fewer nuances [159].

The pathogenesis of MOF in ALF is not nearly clarified. Cy-

Table 4. Clinical studies on serum Gc-globulin in acute liver failure (ALF). Levels are given as ratios compared to normal values.

Authors	Year	Description	N	T-Gc	CR	F-Gc
Lee et al (109)	1985	ALF	14	0.27	NA	NA
Goldschmidt-Cl. et al (96)	1985	ALF	11	0.31	1.9	NA
Goldschmidt-Cl. et al (101)	1988	ALF	7	0.38	~5	NA
Schiødt et al (1)	1995	ACM OA/extreme	8	0.31/0.25	2.2/5.4	NA
Lee et al (97)	1995	ALF (mainly ACM)	47	NA	NA	0.17
Schiødt et al (2)	1996	ALF	94	0.35	3.2	0.26
Wians et al (106)	1997	ALF	20	0.49	NA	NA
Schiødt et al (6)	2001	ACM OA/extreme	15	0.29	4.4	0.17
Schiødt et al (7)	2005	ALF	182	0.26	NA	NA
Antoniades et al (110)	2005	ALF	53	NA	NA	0.12
Schiødt et al (148)	2005	ALF	178	NA	NA	0.25

ACM = acetaminophen. CR = actin complex ratio. F-Gc = free Gc-globulin. OA = on admission. T-Gc = Total Gc-globulin.

Table 5. Prognostic value of Gc-globulin in acute liver failure.

Study	Location	Cutoff (mg/L)	N	Patients	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
Lee et al (97)	London	34	47	Admission	68	68	59	76
(Free Gc)			27	Day 2	100	85	70	100
Schiødt et al (2)	Copenhagen	100	59	N-ACM	79	60	73	68
(Total Gc)			18	ACM	100	53	30	100
Schiødt et al (7) (Total Gc)	US multi-center	80	106	N-ACM	85	43	65	69

N-ACM = nonacetaminophen etiology. NPV = negative predictive value of a test. PPV = positive predictive value of a test. Sensitivity = proportion of positives (here: nonsurvivors or transplanted patients) correctly identified by the test. Specificity = proportion of negatives (here: nonsurvivors or transplanted patients) correctly identified by the test.

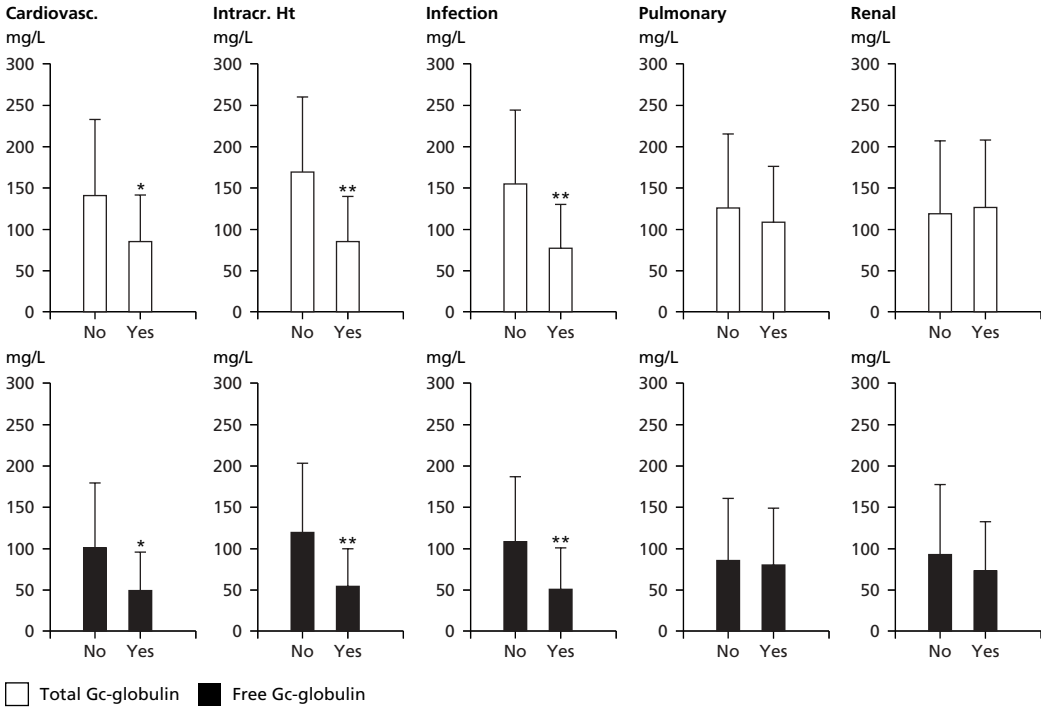

Figure 6. Admission total and free Gc-globulin concentrations and relationship to development of organ failures in patients with grade III and IV acute liver failure. Patients who developed cardiovascular failre, intracranial hypertension, and infections had significantly lower Gc-globulin levels compared to those who did not develop these complications.
*) p < 0.01. **) p < 0.001.

tokines (e.g., IL-6, IL-1, and TNF), endotoxemia, or ischemia have been suggested to be important variables [160]. Lack of Gc-globulin could be one of the factors contributing to the development of MOF in ALF, since this could lead to actin-induced thrombosis resulting in tissue hypoxia, a frequent complication of ALF [161-163]. Seventy-nine patients with ALF and peak hepatic encephalopathy grade III/IV (a subset of the patients reported in study [2]) were studied with respect to admission levels of Gc-globulin and the development of organ failure [3]. The most common organ failure was pulmonary failure, followed by renal failure, infection, cardiovascular failure, and intracranial hypertension. Total and free Gc-globulin levels were significantly lower in patients developing infection, cardiovascular failure, or intracranial hypertension, whereas levels did not differ among patients with or without pulmonary or renal failure (Figure 6).

Patients with Gc-globulin values in the first quintile (lowest 20%) had almost 3 times as many organ failures as patients with values in the fifth quintile (Figure 7). Sixty-five per cent of the patients developed MOF, defined as two or more organ failures (in addition to the hepatic failure and the presence of hepatic encephalopathy). These patients had lower total and free Gc-globulin than patients without MOF.

Is lack of Gc-globulin pathogenetically involved in the development of MOF? It seems highly unlikely that a single mediator should be responsible for all the profound disturbances seen in ALF. Rather, lack of Gc-globulin may be part of the explanation, together with mediators such as TNF, IL-1, IL-6, nitric oxide, and important cells like Kupffer cells, macrophages, endothelial cells, and the immunologic system [160]. Reduced Gc-globulin levels may be suggested to influence the course of illness in two ways: by the formation of (local) ischemia caused by actin thrombi formation, or by increasing the susceptibility to infection via a decrease in the non-specific immune functions of Gc-globulin (table 1).Capillary obstruction may be caused by cellular debris (actin, collagen) from the failing liver. *Bihari et al* demonstrated tissue hypoxia to occur in patients with grade III and IV ALF, evidenced by hyperlactatemia and metabolic acidosis [161;162]. Microvascular disturbances are apparently the main cause of tissue hypoxia, perhaps developing because of arteriovenous shunting [162], reflected hemodynamically as reduced systemic vascular resistance and decreased oxygen extraction ratio [161]. However, lactic acidosis may also stem from accelerated glycolysis [153]. It remains to be studied if actin-containing thrombi are a pathologic feature of ALF.

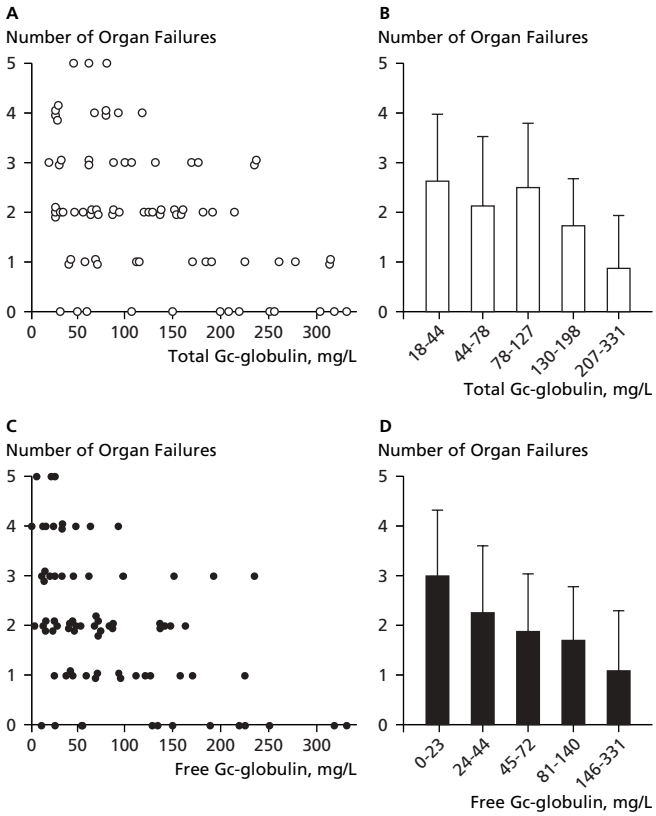

Figure 7. The relationship between admission levels of total and free serum Gc-globulin values and number of organ failures in patients with acute liver failure and hepatic coma grade III or IV (**A** and **C**). **B** and **D**: the quintiles of total and free Gc-globulin vs. number of organ failures. Spearman's rank correlation coefficient was –0.42 (total Gc) and –0.46 (free Gc), P< 0.005 for both.

In conclusion, it is not proven that lack of Gc-globulin/actin toxicity contributes to the development of MOF in ALF. However, even in these extremely sick patients, Gc-globulin levels clearly reflect the

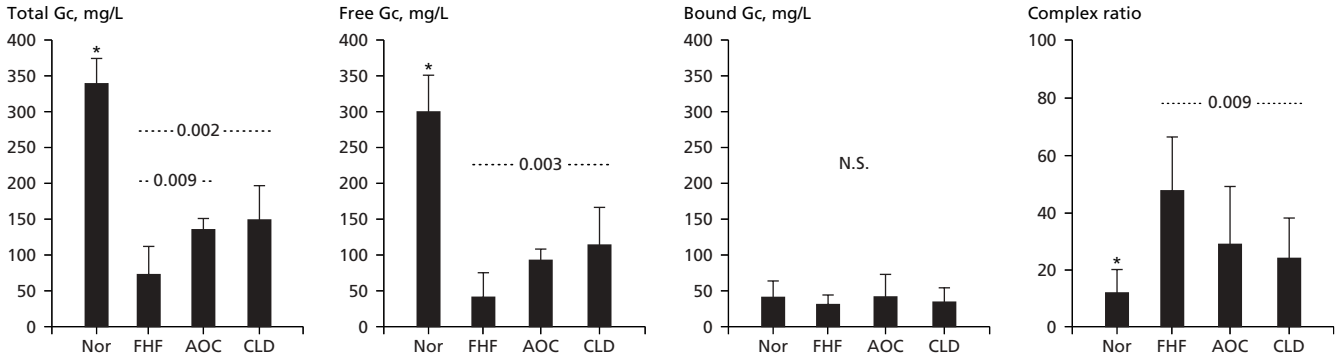

Figure 8. Total, free, and bound Gc-globulin levels and complex ratio in patients with acute liver failure (FHF), acute on chronic liver disease (AOC), and chronic liver disease without coma (CLD) compared with normal controls (Nor).

risk of organ failures. Admission Gc-globulin concentrations can therefore indicate if subsequent MOF develops.

GC-GLOBULIN KINETICS IN LIVER DISEASE

Gc-globulin is almost entirely synthesized in the liver although smaller amounts of Gc-globulin mRNA are also expressed in the kidney, the testis, and the abdominal fat in rats [26]. In normal individuals, Gc-globulin kinetics was studied by *Kawakami et al* using injection of radio-labeled Gc-globulin (^{125}I-labeled Gc) [34]. They found that a three-pool model could best explain Gc-globulin kinetics, pool 1 being plasma, pool 2 the extravascular, extracellular compartment, and pool 3 the intracellular compartment. The total exchangeable Gc-globulin was 2.89 gram and the production rate was 0.69-0.93 g/day with a mean of 0.80 g/day [34].

Protein turnover is generally decreased with advanced liver disease, most so in patients with hepatic coma where protein synthesis is only one third to one half of that observed in normal individuals [164]

Guha et al studied the regulation of Gc-globulin expression *in vitro* in Hep3B hepatocytes and found that interleukin-6 and dexamethasone increased Gc mRNA and secreted protein by twofold whereas TGFβ decreased it by fivefold [165]. IL-1 and TNF did not affect Gc-globulin expression significantly. It is known that plasma levels of IL-6 (along with IL-1 and TNF) are increased in acute liver failure [166]. In an animal study, Gc-globulin mRNA expression increased slightly after inflammation whereas partial hepatectomy lead to a decrease in mRNA levels [167].

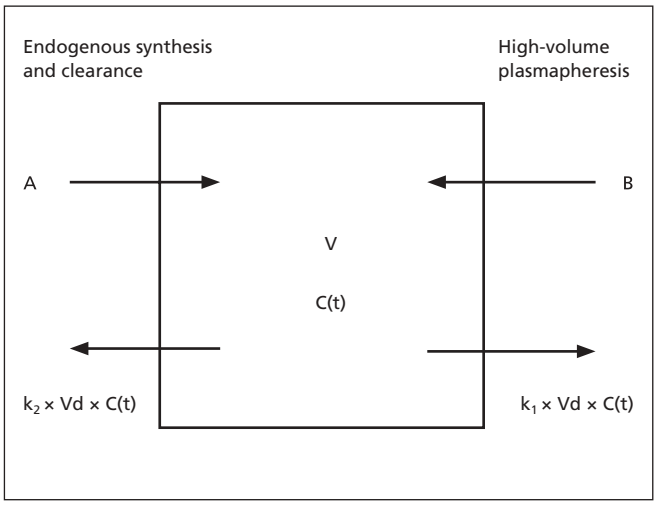

Figure 9. Model for estimation of production rate and halflife of Gc-globulin. A: hepatic production rate of Gc-globulin. Plasma infusion rate during high volume plasmapheresis. C(t): concentration of Gc-globulin at any given time. k_1: rate constant for plasma removal during plasmapheresis. K_2: rate constant for Gc-globulin clearance. Vd: volume of distribution of Gc-globulin.

In study [5], Gc-globulin kinetics were studied in 22 patients with acute and chronic liver disease, all undergoing liver vein catheterization. Total and free Gc-globulin concentrations were lowest in patients with ALF, and patients with chronic liver disease had concentrations approximately 2-fold higher than in ALF (Figure 8), in keeping with the results reported elsewhere in this thesis. No difference in Gc-globulin concentrations in the hepatic vein, the artery, and the central vein was detected even though Gc-globulin is presumed to be almost entirely synthesized in the liver. This apparent discrepancy can be rather easily explained by the inaccuracy of the analytical method.

Most patients with hepatic encephalopathy also underwent high volume plasmapheresis (exchange of 8-10 liters of plasma), a treatment option that may improve survival in some patients with ALF serving as a bridge to urgent liver transplantation [113;168]. An estimate of Gc-globulin production and halflife could be made in these patients assuming a single compartment model and a volume of distribution (Vd) of 6 litres (Figure 9). The Gc-globulin production rate was 4.1 ± 1.3 mg/min – 7-fold higher than the production rate reported in healthy adults in the literature [34]. This surprising observation indicates a high priority for Gc-globulin in the necrotic liver – as opposed to the conditions after partial hepatectomy [167] – and protection against actin toxicity seems like an obvious explanation for this high priority, since actin release from necrotic hepatocytes is so abundant. Also, Gc-globulin's immune functions may be needed in ALF and acute on chronic liver disease, both conditions being characterized by a high proportion of infections [151;169]. Still, Gc-globulin concentrations were low despite the Gc-globulin production increase, and this was due to a shorter than normal halflife of Gc-globulin [5]. The Gc-globulin:actin complex has a much shorter halflife than uncomplexed Gc-globulin [36;59] and the observed decrease in Gc-globulin's halflife is possibly due to increased actin complexing. However, Gc-globulin could also have been consumed as part of its function as a precursor of macrophage activating factor [170;171] or activation of killer cells [83]. A possible bias in our study [5] was the fact that we compared our results in liver patients with normal subjects reported in the literature rather than performing the same measurements in normal subjects. However, the results were so striking that it is very unlikely that this would have changed the conclusions of the study.

Bound Gc-globulin was constant and within normal range in all patients and also before and after high volume plasmapheresis, in keeping with the results reported in [6]. We speculate that uptake and degradation of the Gc-globulin:actin complexes are regulated by bound Gc-globulin concentrations, even in situations of severe liver damage, by a receptor mechanism (Figure 9). The complexes are taken up in sinusoidal endothelial cells or Kupffer cells [58;59] and these cell lines may not suffer as much as hepatocytes during liver injury [172;173] which could mean that the proposed regulatory mechanism is still intact in ALF and severe chronic liver disease. However, proof for this hypothesis is lacking. Also, this hypothesis

may be challenged by the finding of very low (<5%) actin complex ratio levels in one study using crossed immunoelectrophoresis [100] rather than rocket immunoelectrophoresis. This is also what should be expected since the halflife of the Gc-globulin:actin complex is so much shorter than that of uncomplexed Gc-globulin [34;36] – unless of course the proposed hypothesis is correct. Further studies using several methods of analysis in the same serum samples should elucidate these points.

CHRONIC LIVER DISEASE AND LIVER TRANSPLANTATION

Chronic liver disease may arise from a number of etiologies. However, once cirrhosis develops symptomatology and clinical findings are very similar among the patients with the risk of decompensation in the form of variceal bleeding, ascites, hepatorenal syndrome, or hepatic encephalopathy.

Gc-globulin levels in chronic liver disease without cirrhosis have been reported in 5 studies (Table 6) [16;96;104;105;109]. Serum Gc-globulin was normal in 2 of those studies and below normal in the remaining 3; in 2 of them levels were one half of normal. The complex ratio was only measured in one study where *Goldschmidt-Clermont et al* found complex ratio levels to be above normal [96].

Nine studies have reported on Gc-globulin levels in cirrhotic patients (Table 6) [4;5;20;63;103;105;160;174;175] and three of those reported on patients with end-stage liver disease/decompensated cirrhosis [4;5;175]. Total Gc-globulin concentrations were reduced in all studies, to between 45% and 92% of normal levels in compensated cirrhosis and to between 33% and 69% in decompensated cirrhosis. Complex ratio was measured in 2 studies [4;5] and was found to be increased in one of those studies [5]. Free Gc-globulin levels were reported in two studies [4;5] and were below normal range in both of them, in parallel with total Gc-globulin concentrations (Table 6).

Liver transplantation is required for some patients with decompensated cirrhosis [176]. After liver transplantation, Gc-globulin genotype seems to convert to that of the donor [177]. Gc-globulin and complex ratio in patients with end-stage liver disease before and after liver transplantation were reported in one study [4]. A minority of the patients had normal Gc-globulin levels before transplantation. In this group Gc-globulin levels remained normal after transplantation (Figure 11). The majority of the patients had subnormal Gc-globulin levels before transplantation. In this group Gc-globulin levels gradually increased in most patients after transplantation (Figure 11). This course parallelled that of the increase of the prothrombin index but was in contrast to the continuous decrease in albumin levels (Figure 12). So even though albumin and Gc-globulin are phylogenetically closely related they are regulated very differently in the first 2 weeks after liver transplantation, in keeping with the differences in the cytokine regulation of Gc-globulin and albumin synthesis observed in isolated hepatocytes [165]. These findings are in contrast to an animal study where albumin synthesis was found to be normal already 2 hours after liver transplantation [178] and to a study where albumin synthesis was reported to be normal 13 months post-transplantation in humans [179].

In conclusion, Gc-globulin levels are moderately decreased in chronic liver disease, most so in patients with cirrhosis and espe-

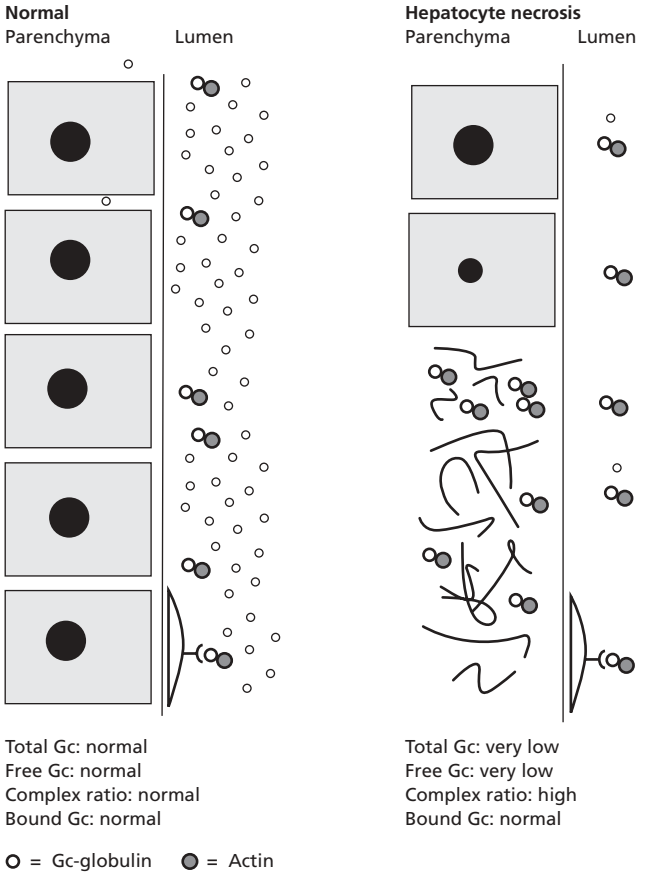

Figure 10. A schematic view of the hypothesized results of hepatic necrosis on Gc-globulin and actin complex ratio. Total and free Gc-globulin concentrations decrease significantly following hepatocyte necrosis, whereas bound (actin-complexed Gc-globulin) remains constant and normal due to a proposed regulatory mechanism in endothelial cells that is intact even in liver failure.

Table 6. Clinical studies on serum Gc-globulin in chronic liver disease. Levels are given as ratios compared to normal values.

Authors	Year	Cirrhosis	Patients	N	T-Gc	CR	F-Gc
Barragry et al (16)	1978	No	CLD (PBC, ALD, CAH)	45	0.78	NA	NA
Bikle et al (104)	1984	No	ALD	25	0.47	NA	NA
Lee et al (109)	1985	No	CLD (PBC, ALD, CAH)	49	~1	NA	NA
Goldschmidt-Cl. et al (96)	1985	No	CAH	7	0.52	1.6	NA
Diamond et al (105)	1989	No	CLD	54	1.06	NA	NA
Bouillon et al (63)	1977	Yes	Cirrhosis	16	0.80	NA	NA
Brown et al (20)	1979	Yes	Cirrhosis	37	0.77	NA	NA
Walsh et al (103)	1982	Yes	Cirrhosis	4	0.65	NA	NA
Constans et al (19)	1983	Yes	Alcoholic cirrhosis	17	0.62	NA	NA
Bouillon et al (174)	1984	Yes	Cirrhosis	32	0.92	NA	NA
Masuda et al (175)	1989	Yes	Cirrhosis	8	0.67	NA	NA
			Decompensated cirrhosis	14	0.33	NA	NA
Diamond et al (105)	1989	Yes	Cirrhosis	53	0.88	NA	NA
Schiødt et al (4)	1999	Yes	ESLD	17	0.69	1.1	0.67
Schiødt et al (5)	2001	Yes	Cirrhosis	8	0.45	1.9	0.35
			Cirrhosis with HE	4	0.39	2.4	0.32

ALD = alcoholic liver disease. CAH = chronic active hepatitis. CLD = chronic liver disease. CR = complex ratio. ESLD = end-stage liver disease. F-Gc = free Gc-globulin. HE = hepatic encephalopathy. PBC = primary biliary cirrhosis. T-Gc = total Gc-globulin.

cially in decompensated cirrhosis. Future studies should elucidate if this decrease is caused by a fall in Gc-globulin production or an increased Gc-globulin clearance – or both. Gc-globulin concentrations normalize in most patients within the first 2 weeks following liver transplantation, in contrast to albumin concentrations, indicating a higher priority for Gc-globulin.

OTHER ASPECTS OF THE EXTRACELLULAR ACTIN-SCAVENGER SYSTEM IN DISEASE

Serum Gc-globulin levels have been studied in other conditions than liver diseases (Table 7).

The degree of reduction of Gc-globulin levels in disease seems to correlate with both the amount of necrosis and the cytokine response (systemic inflammatory response syndrome, SIRS), since the lowest levels have been observed in septic shock [180], a condition characterized by widespread necrosis and hyperactivation of SIRS [192-194]. Moderate reductions of serum Gc-globulin have been observed in multiple trauma [163;185-187], in which SIRS is less activated than in septic shock [194]. The decrease in Gc-globulin concentrations in trauma can been observed as early as 45 minutes after injury [185] and Gc-globulin levels have prognostic value also in this condition [186]. The reduced levels in nephrotic syndrome are caused by urinary loss of Gc-globulin [184].

Interestingly, pregnancy (especially late pregnancy) seems to induce an increased synthesis of Gc-globulin [16;63;96;100;103;104], possibly due to increased estrogen levels, as estrogen therapy causes increased Gc-globulin levels [16]. For unknown reasons short bowel syndrome patients also have increased serum Gc-globulin values, (*Schiødt et al*, data not published).

The other protein of the extracellular actin scavenger system, gelsolin, has been studied in a number of diseases (Table 8).

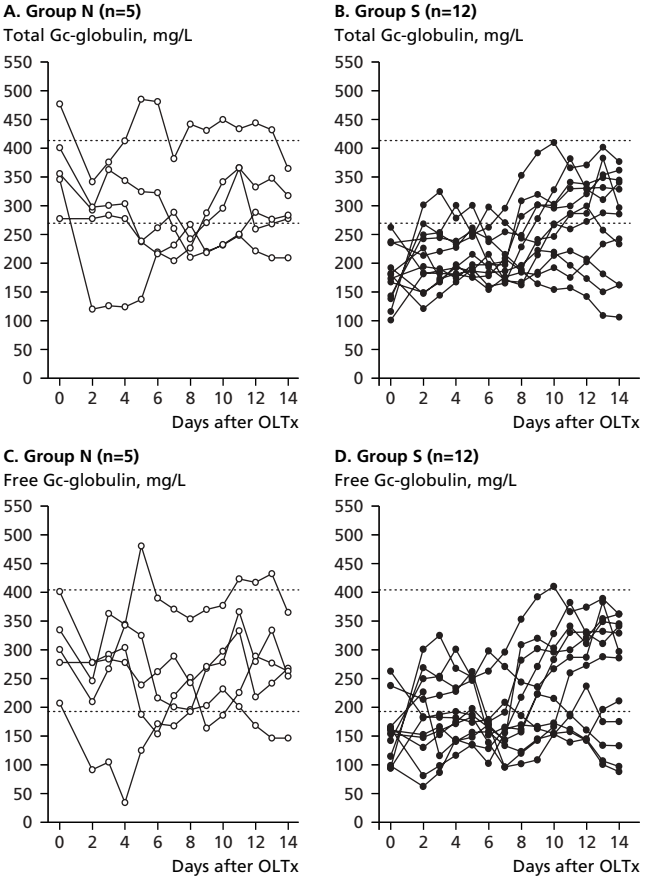

Figure 11. Total (**A, B**) and free (**C, D**) serum Gc-globulin levels immediately before liver transplantation (day 0) and on day 2-14 post-transplantation. Group N: had normal total Gc-globulin before transplantation (**A, C**). Group S: had subnormal total Gc-globulin before transplantation (**B, D**). Dotted lines represent normal range (nl).

All the diseases mentioned in Table 8 – except cancer – involve acute cellular necrosis. Serum levels of gelsolin seem to correlate with the degree of disease severity and the lowest levels have been observed in ALF. It is not known if the reduction of serum gelsolin concentrations is related solely to an increased consumption of gelsolin due to actin scavenging or maybe also to decreased gelsolin production.

Thus, in diseases and conditions involving tissue necrosis or tissue injury the two components of the extracellular actin scavenger system, Gc-globulin and gelsolin, are invariably affected and serum levels of the 2 proteins are reduced, most so in patients with ALF or septic shock. It remains to be studied if this reduction may be pathogenetically involved in the diseases. One very recent study (208) suggests so, since infusion of recombinant gelsolin to endotoximic mice improved survival significantly.

PERSPECTIVES:

The papers described in this thesis confirm that the actin scavenger

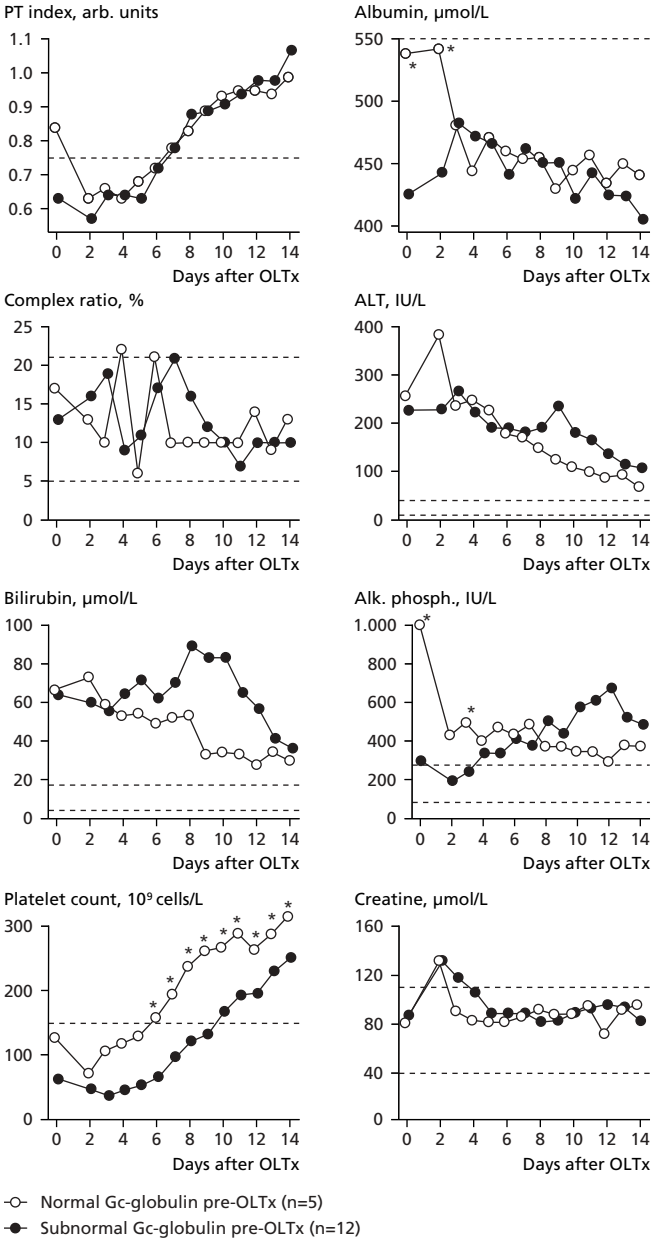

Figure 12. Mean values of biochemical markers immediately before liver transplantation and on day 2-14 post-transplantation. Dotted lines represent normal range (mean ± 2 SD) (nl). *) P< 0.05 between groups. ALT: alanine aminotransferase. Alk. phosph: alkaline phosphatase. PT index: protrombin index (activity of coagulation factors II, VII; and X).

Table 7. Serum Gc-globulin in non-hepatic diseases.

Condition (ref.)	Serum Gc-globulin
Septic shock (180;181)	↓↓
Prematurity (182;183)	↓
Nephrotic syndrome (184)	↓
Multiple trauma (163;185-187)	↓
Post-operatively (herniorrhaphy) (188-190)	↓
Ischemic heart disease (*Hanash et al*, data not published)	↓
Gastrointestinal diseases (191)	=
Pregnancy (16;63;96;100;103;104)	
First trimester	=
Second trimester	(↑)
Third trimester	↑
Estrogen therapy (16;63)	↑
Vitamin D deficiency (16)	↑
Short bowel syndrome (*Schiødt et Jeppesen*, data not published)	↑

Table 8. Serum gelsolin in various diseases.

Condition (ref.)	Serum gelsolin
Acute liver failure (51;195-197)	↓↓
Malaria attack (198;199)	↓
Trauma (200;201)	↓
Cancer (202-204)	↓
Acute lung injury (205)	↓
Pneumatic syndrome (206)	↓
Rhabdomyolysis (207)	↓
Septic shock (197)	↓

system is significantly affected in liver disease. Serum levels of Gc-globulin are often markedly reduced and the reduction correlates with the severity and the acuity of the disease, since Gc-globulin concentrations were lowest among patients with ALF, a condition characterized by massive hepatic necrosis. This supports that Gc-globulin is consumed upon tissue necrosis with concomitant actin release. Gc-globulin levels could also be reduced if Gc-globulin were used in its known nonspecific immune functions. This seems very probable since liver diseases are characterized by a high proportion of infections. Future research should try to elucidate Gc-globulin's role in infected liver patients in more detail.

Admission serum Gc-globulin was demonstrated to be of equal value as the King's College Hospital criteria in determining outcome in ALF. The advent of rapid methods of analysis (e.g., immunonephelometry and ELISA techniques) suggests that measurement of serum Gc-globulin could have value for the clinicians taking care of patients with ALF, acetaminophen intoxication, or acute on chronic liver disease. Also, there are a number of severe hepatic conditions with a high mortality where the role of Gc-globulin has not been studied yet, e.g., alcoholic hepatitis or spontaneous bacterial peritonitis.

The key question is really if Gc-globulin – or lack of Gc-globulin – is in any way related to the pathogenesis of severe liver failure. Support for this hypothesis can be found in the observation that even in patients with the deepest grades of hepatic encephalopathy admission Gc-globulin levels had a highly significant predictive value for the later development of organ failure and MOF. Thus, lack of Gc-globulin (or gelsolin) could lead to enhanced tissue necrosis due to either local or systemic actin thrombi formation and to a greater risk of infections, increasing the risk of organ failure and hence death. If this was true, then Gc-globulin substitution should prove valuable. In fact, Gc-globulin has been purified in large scale from plasma Cohn fraction IV (209) and phase I trials on Gc-globulin infusion are currently being planned. Hopefully, a Gc-globulin substitution trial in liver disease patients can be performed within a few years.

APPENDIX

ROCKET IMMUNOELECTROPHORESIS OF SERUM GC-GLOBULIN

Serum levels of Gc-globulin was measured by rocket immunoelectrophoresis in 6 of the 7 studies used in this thesis [1-6]. Therefore, the appendix describes this method in detail.

Blood samples were collected from either a cubital vein, a central vein (usually the superior caval vein, via a central venous line), or a peripheral artery (the radial artery). Blood samples were placed at room temperature for one and a half hour to allow clot-retraction, hereafter refrigerated at +4°C for at maximum of 3 days, usually for less than 24 hours. The serum was separated at 3,000 RPM and stored at -20°C until analysis. We studied the stability of Gc-globulin in serum samples, stored at -20°C for up to 15 years in 21 patients admitted to this Department between 1978 and 1980 with no sign or biochemical evidence of liver disease or other disease. These patients were typically admitted for evaluation of potential intoxication or Gilbert's syndrome. Gc-globulin levels in this group did not differ from normal individuals [2].

We adapted the Gc-globulin analysis from *Goldschmidt-Clermont et al* [96] to provide an accurate rocket immunoelectrophoresis method for determining both serum Gc-globulin and the percentage of Gc-globulin complexed to G-actin (i.e., complex ratio). The principle of rocket immunoelectrophoresis is precipitation of antibody and antigen in an antibody containing agarose gel, over which an electrical field is being applied [210]. We applied 30 mL of 1 per cent w/v agarose (Agarose Litex HSA, Bie & Berntsen) gel (56°C) containing 300 µL polyclonal anti-human Gc-globulin (DAKO, Glostrup, Denmark) on a 200 × 100 mm glass plate to form a uniform 1 mm agarose layer on the plate. Twenty-six small wells were punched after setting of the gel. Five µL of a sample were added in each well. The glass plates were placed in the electrophoresis box and connected to the two trunks, filled with TRIS-Veronal buffer (0.02 M, pH 8.6), via five layers of filter paper wicks (Frisenette, Ebeltoft, Denmark). Separated by filter paper wicks, three agarose containing glass plates were placed on top of each other in each electrophoresis box. A glass plate was placed on the upper wicks to avoid water condensation on the surface of the gel. Normally, six agarose gels were used per day in the laboratory. An LKB power supply type 3371 C generated the sufficient field strenght, 10 mV/4 cm, and the water-cooled electrophoresis ran overnight at a temperature of 14 °C. To remove non-precipitated proteins following electrophoresis, the gels were pressed (45 minutes), washed in destilled water for 10 minutes, pressed again (45 minutes), and hot air-dried, using a hair-drier, until only a very thin »gel-film« remained on the glass plate. The films were stained in a Coomassie Brilliant Blue R-250 (Sigma B-0149) solution (Coomassie Brilliant Blue 5 g; ethanol 96%, 450 mL; glacial acetic acid, 100 mL; distilled water, 450 mL) for 15 minutes and destained in the destaning solution (same ingredients as above, except for Coomassie) for 10 minutes.

The height of the rockets, as measured from the top of the well to the tip of the rocket, is proportional to the amount of antigen in the well.

The electrophoretic mobility of the Gc-globulin:G-actin complex has been shown to be greater than that of Gc-globulin alone [96;211]. Therefore, two sets of known concentrations of Gc-globulin (human Gc-globulin, SIGMA CHEMICALS CO, USA, code: G-6889) were applied on each gel: one with Gc-globulin alone, and one with Gc-globulin and saturating amounts of actin (porcine actin, SIGMA CHEMICALS CO, USA, code: A-0541). Five different Gc-globulin concentrations were used for each standard curve: 1/6.75, 1/11, 1/18, 1/30, and 1/50. The samples were diluted 1:2 (one part sample, 2 parts isotonic saline or actin solution) before application in the wells. The gels contained two sets of standards and 4 patient samples (each with and without actin added, both in duplicate). The two standard curves were plotted in a double logarithmic

Table 9. The results (mean, median, range) of *standard curve* linear regression analysis from fifty consecutive rocket immunoelectrophoresis plates, analyzed between April and August 1995. The equation for a line is: y = constant + αx. Values for standard curves without and with actin were compared with Mann-Whitney's rank sum test (Normality test failed for all groups). SE = standard error.

	CONSTANT	SE of y	R^2	α	SE of α
Standard curve without actin					
Mean	-0.931	0.081	0.991	0.958	0.051
median	-0.862	0.078	0.991	0.945	0.049
range	(-2.497-(-0.181)	(0.047-0.143)	(0.980-0.998)	(0.808-1.246)	(0.030-0.090)
Standard curve with actin					
Mean	-0.034	0.040	0.996	0.852	0.026
median	-0.072	0.035	0.997	0.862	0.026
range	(-0.535-0.591)	(0.009-0.077)	(0.988-1.000)	(0.710-0.946)	(0.005-0.049)
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

diagram. The total Gc-globulin concentration in a sample was calculated by plotting the natural logarithm (ln) versus the rocket height of the sample with added actin in the diagram and using the upper standard curve. A perpendicular line from this point on the Standard + actin curve was drawn. Next, the ln (rocket height of the sample with no added actin) was plotted in the diagram, and the junction between this value and the perpendicular of the Gc-globulin + actin was noted. The complex ratio was calculated as the ratio y/(y + x). A semiautomated computer program (in the software program ®Lotus 1-2-3 version 2.3 for DOS) was constructed to calculate serum Gc-globulin and complex ratio. The two standard curves were calculated using linear regression analysis. Table 9 shows the results of fifty consecutive standard curves. Both standard curves had mean and median R^2 values over 0.99, highest for the standard curve with actin. The slope of the curve was steeper for the lower standard curve, in accordance with the original description (96). Standard error for the slope was in the 2 to 5 per cent range for both curves, whereas standard errors of Y was 4 % and 8 %, respectively. Repeated analyses of a given sample yielded a 6 % difference of the calculated serum Gc-globulin value (coefficient of variance), and a little higher difference for the calculated complex ratio.

SUMMARY

ENGLISH

This Doctoral thesis is based on 7 previously published papers and reports on the role of the actin-scavenger Gc-globulin in acute and chronic liver diseases. Gc-globulin is synthesized in the liver and is a multifunctional protein; however, its main physiologic function is presumably actin binding and actin scavenging. Actin is a major cellular protein released during cell necrosis that may cause fatal formation of actin-containing thrombi in the circulation if the actin scavenging capacity of Gc-globulin is exceeded.

In my studies, I found serum Gc-globulin levels to be reduced in liver disease, most so in patients with acute liver failure (ALF). In patients admitted with acetaminophen (paracetamol) overdose, Gc-globulin concentrations were lower in patients with hepatic encephalopathy than in those without and the levels nadired at approximately 60-72 hours after acetaminophen ingestion, corresponding with the peak in aminotransferese levels (and thus, hepatic necrosis).

In patients with ALF, admission Gc-globulin was significantly lower in 47 nonsurvivors than in 30 survivors, 26% and 46% of normal, respectively (P< 0.001). The predictive value of outcome using a Gc-globulin cutoff level of 100 mg/L equaled that of the internationally accepted King's College Hospital criteria. The prognostic value of Gc-globulin was confirmed in a separate study including 106 patients from the United States with nonacetaminophen-induced ALF now using an automated nephelometric assay whereas the prognostic value seemed less obvious for acetaminophen-induced ALF.

Multiple organ failure (MOF) is a frequent complication of ALF. In ALF patients with deep coma (hepatic encephalopathy grade III or IV) Gc-globulin levels correlated inversely with the number of failing organs. Levels were lower in patients who later developed MOF than in those who did not.

Surprisingly, kinetic studies in patients with ALF and acute on chronic liver disease showed Gc-globulin production to be 7-fold increased in these conditions. Despite this increase Gc-globulin levels were severely reduced and the reduction must therefore be due to a highly increased consumption of Gc-globulin - probably because of hepatocyte necrosis and removal from the circulation of Gc-globulin:actin complexes or because of its role in immune-related functions.

Patients with chronic liver disease had reduced Gc-globulin levels, but the reduction was less pronounced than in ALF. After liver transplantation, Gc-globulin concentrations normalized within 2 weeks, in contrast to the continuous decrease in albumin levels suggesting a very different regulation of these 2 phylogenetically related proteins.

It remains to be studied if lack of Gc-globulin contributes to the pathogenesis of patients with ALF or chronic liver disease. Future studies should focus on the potential value of Gc-globulin substitution in these patients.

Abbreviations used: ALF: acute liver failure. ELISA: enzyme linked immunosorbent assay. MOF. Multiple organ failure.

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