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A retrospective cohort study of patients with eosinophilia referred to a tertiary centre

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ABSTRACT

Introduction. Patients with eosinophilia (an increased number of eosinophilic granulocytes > 0.5 × 10^9/l in the blood) are encountered in all medical specialties and frequently need thorough workup to identify the eliciting causes and decide whether treatment is indicated. In Denmark, highly specialised centres for eosinophilic diseases or conditions have been established to provide a foundation for the management of complicated cases. Here, we present experiences from such a multidisciplinary centre.

Methods. This was a retrospective study of all patients seen in our tertiary centre for eosinophilia in the 2016-2019 period.

Results. Referrals mainly derived from specialised secondary care and to a lesser degree from primary care physicians. Patients were either asymptomatic or exhibited symptoms from up to three organ systems and presented a median eosinophil count of 1.7 × 10^9/l. Up to eight new clonality analyses or imaging studies per patient were performed after referral. One of these, T-cell receptor analysis, was performed frequently but provided limited information, whereas, e.g., flow cytometry proved more clinically applicable owing to its broader diagnostic range. In total, 51 patients were evaluated and classified as secondary (59%), myeloid neoplasm with *PDGFRA* rearrangement (2%), idiopathic hypereosinophilic syndrome (31%) and idiopathic hypereosinophilia (8%).

Conclusion. The value of a multidisciplinary and versatile approach in a highly specialised centre has a positive impact on diagnostic processes as well as on the evaluation of treatment need.

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Trial registration. not relevant.

Eosinophilia (eosinophil count > 0.5 × 10^9/l blood) represents a clinical challenge that frequently requires detailed information about symptoms and a thorough examination combined with adequate diagnostic workup [1-3]. The incidence in the hospital setting varies by medical specialty and geographical region, reflecting the broad clinical spectrum of conditions associated with eosinophilia [4-6]. Eosinophilia was registered in 4% of all

differential blood sample counts requested by general practitioners in unique, adult individuals in the Capital Region of Denmark during a ten-year period [7]. The eosinophilic granulocyte (eosinophil) possesses numerous physiological functions protecting against helminths and other infections and is involved in homeostatic activities [8]. However, uncontrolled activity of off-target eosinophils or constituents released from their granules may cause mono- or multiorgan dysfunction affecting any tissue [9, 10]. Three levels of eosinophilia have been arbitrarily defined; mild: 0.5-1.5 × 10^9/l, moderate: ≥ 1.5 × 10^9/l and severe: > 5 × 10^9/l. The specific term "hypereosinophilia" commonly refers to moderate or severe eosinophilia.

Eosinophilia is categorised as *primary* when reflecting a clonal haematological condition in which the production of eosinophils is driven by a genetic cause, intrinsic in haematopoiesis; and as *secondary* when the increase is reactive to factors with an extrinsic impact on the eosinopoiesis [1-3]. *Secondary/reactive* eosinophilia is driven, in particular, by the T-lymphocyte-produced cytokine interleukin (IL)-5 and is related to the differentiation and activation of eosinophils in interplay with other immunologically active cells associated with autoimmunity, inflammation, infection, malignancy and allergy including adverse reactions to various drugs [1, 5, 8, 11]. When neither primary nor secondary causes are demonstrable, patients with persisting eosinophil counts of at least 1.5 × 10^9/l are categorised as idiopathic hypereosinophilia (iHE, without symptoms) or idiopathic hypereosinophilic syndrome (iHES, with symptoms) [1-3], although clonality is likely involved in the pathobiology [12].

In the clinic, however, it may prove difficult to disentangle the diagnosis of iHES, primary or secondary eosinophilia due to the rarity of the syndromes and to overlapping manifestations including a heterogenous array of symptoms. Therefore, centralisation of management in tertiary referral centres has been established. The Centre for Eosinophilia (CEOS) is part of the Department of Haematology X, Odense University Hospital, and part of a National initiative for tertiary centres, since 2017 [3]. Patients are referred to CEOS for further diagnostic workup and for second opinion in case of unexplained eosinophilia. According to the national organisation of this tertiary centre in eosinophilia, collaboration within the CEOS encompasses general practitioners, departments at the Odense University Hospital or other departments at hospitals in the Southern Region of Denmark, five departments of haematology localized in the Region of Southern, Central or Northern Denmark (3.11 million inhabitants).

The aim of this retrospective observational study, based on cross-sectional analyses of adult patients referred to our multidisciplinary centre during a three-year period, aimed to benchmark our current performance with respect to diagnostic processes and treatment decisions.

METHODS

Patients were included in the study if scheduled for an appointment in the CEOS during the study period. Records of telephone conferences without a subsequent referral were unavailable. Each patient was identified by the International Classification of Disease-10 codes: hypereosinophilic syndrome (DD475), eosinophilia (DD721), eosinophilic lung infiltrates (DJ829), eosinophilic esophagitis (DK209D) and eosinophilic gastroenteritis (DK528B).

The diagnostic workup at our centre was individualised and related to history, information captured in previous tests, the clinical examination and a pre-planned paraclinical programme in line with guidelines [1-3]. However, diagnostic tests, which had been adequately performed prior to referral, were not repeated. The diagnostic tests, performed at the CEOS, included imaging, cytogenetics and tissue biopsies. Data for the study for each patient were extracted from the file at the time of the first visit in the CEOS, which in some cases occurred in the years leading up to the study period or in new referrals during the study period. All information was anonymised.

Chromosomal analysis and PCR were performed by standard techniques and next-generation sequencing by a commercial assay for rearrangements and mutations in myeloid neoplasms (Ion Torrent Oncomine Myeloid panel/Ampliseq AML research panel (Thermo Fisher)). The PCR method for T-cell receptor (TcR) status and interpretation was employed following European guidelines [13]. The quantitative PCR for *KIT*^{D816V} mutation was performed as described [14]. Flowcytometry was performed including a panel of monoclonal antibodies to detect myeloid (including eosinophils) or lymphoid (including T-cells) proliferation according to Euroflow [15]. Plasma-IL-5 is not a routine analysis at our centre and was not included in our workup. CEOS multidisciplinary conferences were held at 4-6-week intervals; *ad hoc* conferences between specialists as needed, including clinical pathology or radiology, in order to discuss diagnosis, results, treatment and further planning.

The study was approved as a quality assurance project at Odense University Hospital, University of Southern Denmark, in November 2019. Approval from the Ethical Committee was unnecessary under Danish law.

Trial registration: not relevant.

RESULTS

A total of 51 patients were seen at the CEOS between 1 December 2016 and 31 December 2019. A total of 25 of these (49%) were evaluated for the first time in the 37-month study period, whereas the remaining patients had previously been diagnosed with eosinophilia and were seen for a follow-up during the study period. The gender distribution was 55% males (median 54.5 years, range: 18-84 years) and 45% females (median 53.0 years, range: 19-77 years) at the first visit. The duration of known eosinophilia was < 1 month in 10%, < 6 months 25%, < 12 months 18% and more than 12 months in 47% of the study population.

The results of blood cell counts at the first visit are presented in **Table 1**. A total of 16 patients (31%) had received glucocorticoids (GC) before their first visit. Such treatment often normalises the eosinophil count. **Figure 1** presents the symptoms associated with eosinophilia in the population at the first visit in the CEOS. The most frequent symptoms were respiratory in 19 patients (37%, e.g. cough, dyspnoea, nasal secretion) and haematological in nine patients (18%, e.g. B-symptoms including weightloss, unexplained fever or nightsweats, thrombosis or lymphadenopathy); and 13 (25%) patients were asymptomatic. Additional complaints included skin (rash, itching), gastrointestinal (stool change, nausea), rheumatic (joint and muscle symptoms), neurologic (neuropathy, dizziness) and cardiac manifestations (angina, cardiac insufficiency) or oedema. In total, 67% of patients were a- or monosymptomatic, 25% exhibited symptoms from two organ systems and 8% from three organ systems.

TABLE 1 Values of routine analyses of all patients at their first visit in the Centre for Eosinophilia (N = 51).

	Analysis in blood						
	haemoglobin concentration, mmol/l		count, × 10^9/l				
	males	females	white blood cell	eosinophil granulocyte	neutrophil granulocyte	lymphocyte	platelet
Result							
Median (min.-max)	9.1 (7.0-10.6)	7.5 (5.5-9.3)	10.1 (4.9-54.3)	1.7 (0.05-48.9)	5.3 (0.43-15.1)	1.9 (0.3-4.8)	284 (50-598)
Normal range	8.3-10.5	7.3-9.5	3.5-8.8	< 0.5	1.5-7.5	1.0-4.0	145-450

FIGURE 1 Patient symptoms frequency registered at first visit in the centre.

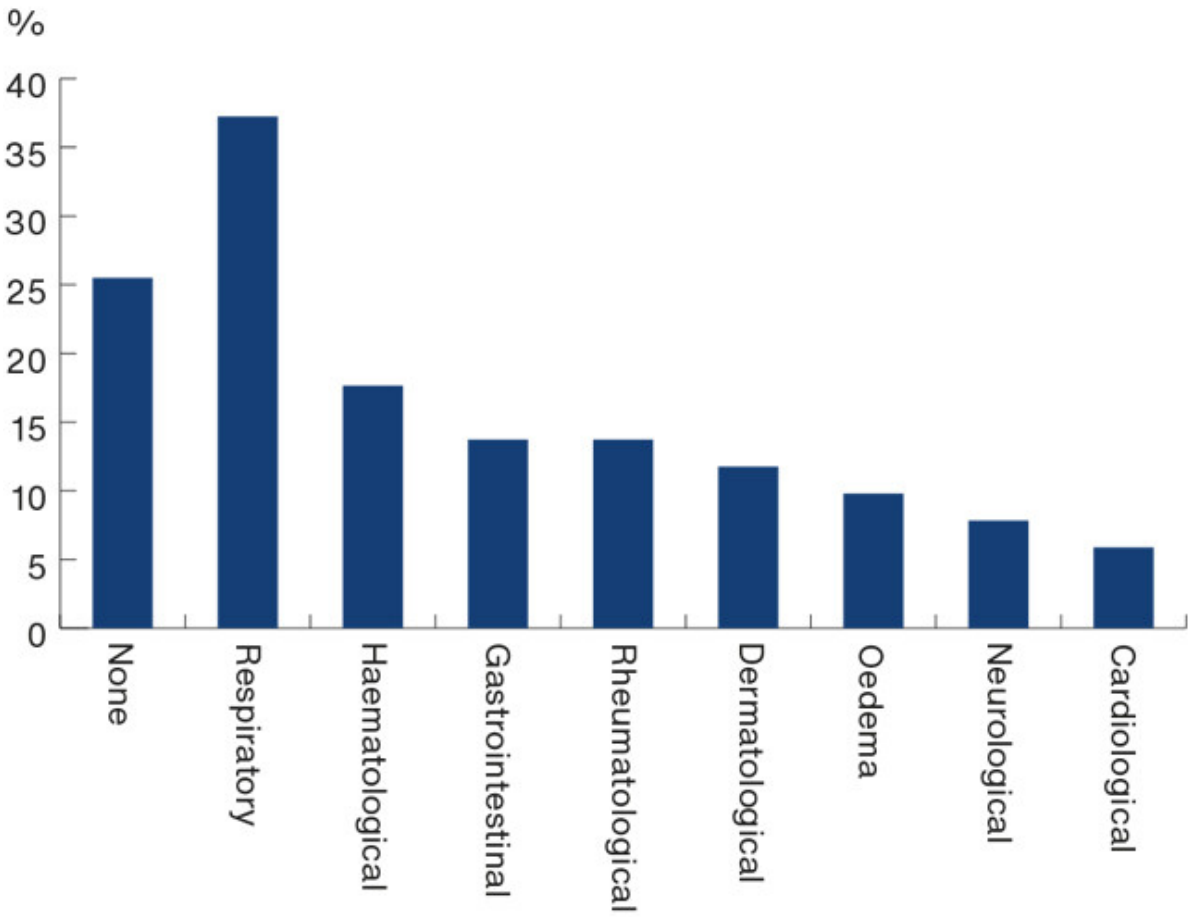
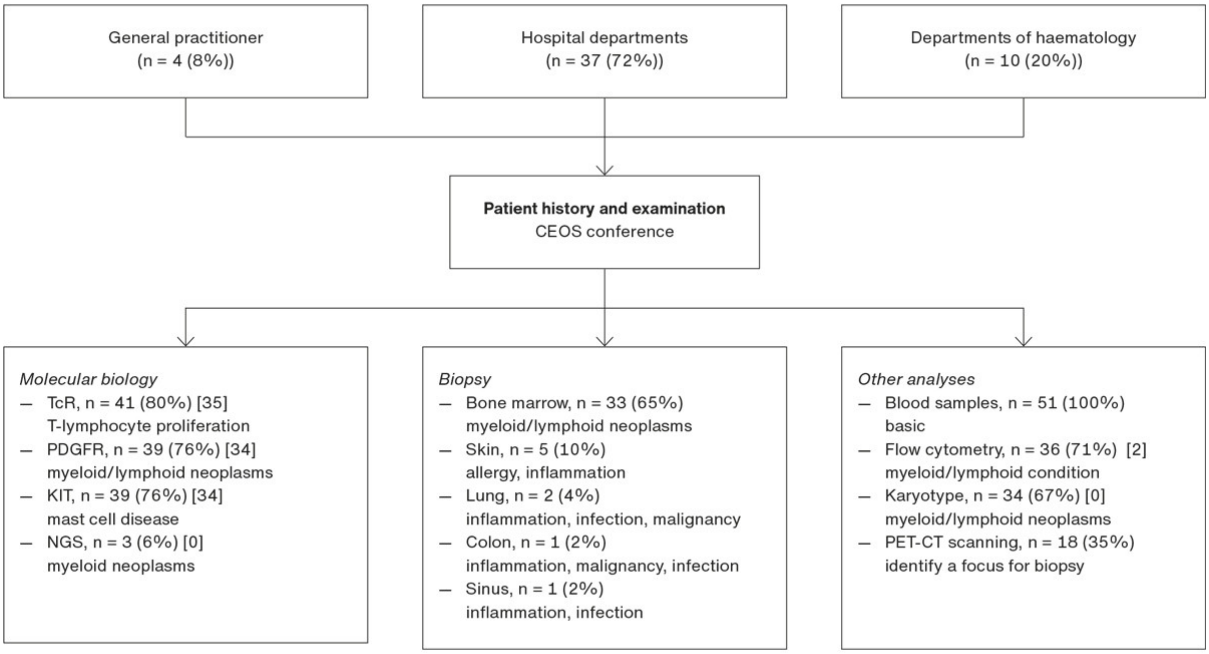


Figure 2 presents a diagram of the patients' referral patterns, listing types and number of analyses performed.Most patients were initially evaluated at another hospital department and only a minor proportion was scheduled for assessment upon request by a general practitioner. The number of specialised analyses performed in the centre *per* patient after referral was: 0-3 (28%), 4-6 (43%), 7-8 (29%).

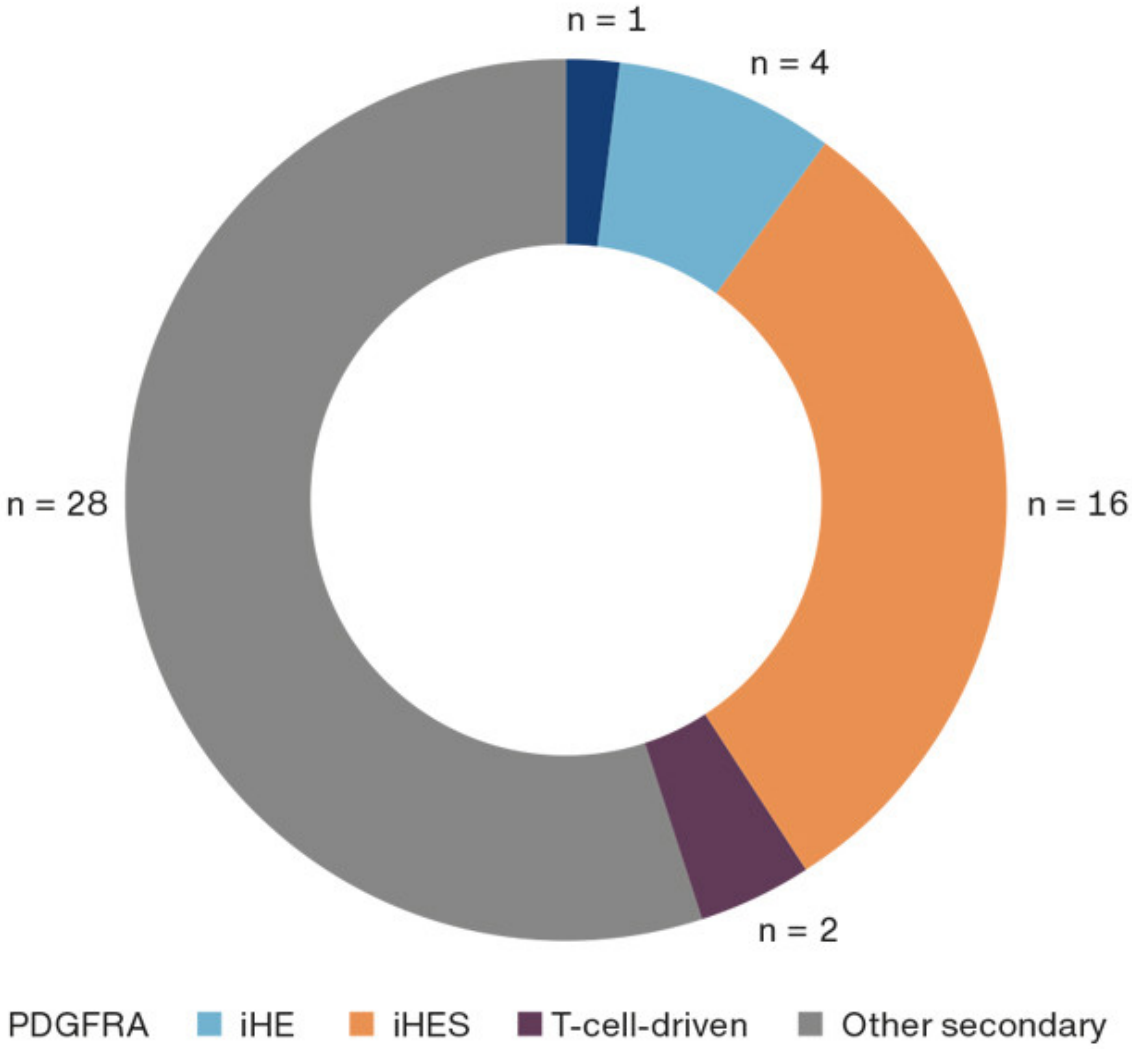
FIGURE 2 Flow diagram of diagnostic work-up of the 51 patients.
The rationales of the analyses and the procedures are stated.



[n]: blood analyses; CEOS = Centre for Eosinophilia; KIT = CD117; NGS = next-generation sequencing; PDGFR = platelet-derived growth factor receptor rearrangement;TcR = T-cell receptor.

Causes of eosinophilia are depicted in **Figure 3**. One patient had a *FIP1L1-PDGFRA* myeloid neoplasm with eosinophilia, representing primary eosinophilia. In all, 30 patients (59%) had secondary eosinophilia, including one patient with systemic mastocytosis associated with acute myeloid leukaemia with the KIT^{D816V}-mutation and del(20q) in the bone marrow, and two patients were considered to have T-cell-driven eosinophilia by TcR and flow-cytometry clonality [16]. The remaining 27 patients had secondary eosinophilia associated with autoimmunity or allergy (eight patients, including rheumatoid or juvenile idiopathic arthritis), inflammation (11 patients, in particular asthma), infection (one patient with bacterial infection and coeliac disease), eosinophil pneumonia (three patients) or iatrogenic (drug-induced, four patients) conditions. The iHES represented a major population, iHE a minor fraction. Several patients had more than one diagnosis potentially explaining secondary eosinophilia (Figure 3).

FIGURE 3 Frequency of causes of eosinophilia in the study population. The number of patients in each diagnostic entity is given (N = 51).



iHE = idiopathic hypereosinophilia; iHES = idiopathic hypereosinophilic syndrome; PDGFRA = rearranged myeloid neoplasm with eosinophilia.

Treatment was chosen as appropriate in all patients, e.g. imatinib in *FIP1L1-PDGFRA* myeloid neoplasm with eosinophilia, and in secondary eosinophilia depending on the underlying condition [1-3]. In some cases, ongoing therapy was re-evaluated to improve the effect. New treatment modalities involved immunosuppression, e.g., methotrexate, mycophenolate and mepolizumab (anti-IL-5) antibody as monotherapy or in combination, also with GC. Patients with iHES were offered myelosuppression (hydroxyurea) or immunomodulatory treatment (alfa-interferon, mycophenolat) in addition to GC. Patients with iHE were not started on cytoreductive therapy according to international guidelines but were merely observed. Patients with iHE discontinued treatment if their treatment had been initiated before their referral to the CEOS. Eleven patients (22%) were discontinued

from CEOS during the study period; the majority for follow-up at a specialised department due to secondary eosinophilia.

DISCUSSION

This study highlighted the versatile activity in a dedicated multidisciplinary forum established to manage complicated cases with eosinophilia, occasionally known for years, and presenting with none, one or several symptoms. Patients were most frequently referred by specialised departments for a second opinion, but also by general practitioners. Hypereosinophilia is captured in primary healthcare and unexplained cases justify interaction with a specialised department due to the variety of diagnoses [7]. The various causes of eosinophilia cannot be disentangled simply based on the symptoms and a clinical examination, and a rich repertoire of paraclinical analyses is accessible to meet the need for diagnostic clarification (Figure 1-3) [1-3].

The eosinophil count ranged from normal values to severe eosinophilia but overall had only a limited impact on the B-haemoglobin level, white blood cells or platelet counts at the first visit in the centre (Table 1).

The diagnostic workup in CEOS adheres to international guidelines [1-3] and ensures that patients and relatives receive adequate information, and aimed at improving the patient's quality of life by optimizing symptom control via a shared decision-making regarding the treatment choice. This study demonstrated that tests for clonality, like TcR, *KIT*^{D816V} mutation analysis and flow cytometry, were performed in most patients. However, the tests contributed to diagnostic clarification only in very few cases. However, exclusion of a clonal condition conveys information, e.g. supporting a diagnosis of iHE or iHES. Besides chromosome analysis, all other tests for clonality may be performed with a similar sensitivity on peripheral blood, which is a logistical advantage.

Flow cytometry proved more clinically applicable owing to its broad diagnostic range [15]. Still, the decision to apply additional diagnostic tests in the individual patient is a delicate balance at the first visit (Figure 2 and Figure 3). No cases of parasitic infections or solid tumour were demonstrated, which may reflect that these cases were identified prior to referral. The majority of patients had secondary eosinophilia [7, 17]. Even so, iHES was common among our patients in contrast to the rarity of this condition in eosinophilia *per se* [1-3, 5]. The explanation is the patient selection at our centre, which serves to justify a centralised function in eosinophilia (Figure 3). One case with a *PDGFRA* rearrangement was identified, which has an excellent prognosis on targeted treatment [18, 19].

One drawback of this retrospective study is the challenge associated with recording all relevant tests performed prior to the first visit. An advantage in an observational study with follow-up is the clarification of the diagnosis.

Patients in this study population with eosinophilia presented with heterogeneous symptoms, as also reported in other studies, emphasizing the risk for (irreversible) organ damage (Figure 1) [6, 17]. This risk is not proportional to the number of circulating eosinophils in peripheral blood [10]. Our findings support that asymptomatic patients with eosinophilia should be referred for specialised assessment due to the complexity of causes. A total of 25% patients were registered as asymptomatic (Figure 1), which is mostly explained by cases with iHE and patients who had received systemic GC before their first visit. Initiating GC may be indicated to achieve rapid symptom relief, but GC may blur a potential positive focus on PET and hamper histological assessments. Hence, initiation of GC should be discussed with haematologists when doubt exists as to the underlying cause.

The multidisciplinary team approach including subspecialised expertice affects our attitude towards the diagnostic process and our view of the need for treatment. Asymptomatic patients do not need treatment and should instead be monitored. Furthermore, the collaboration between colleagues from specialised clinical and diagnostic departments who can meet regularly facilitates a personal contact for ad-hoc conferences concerning

individual patients. Finally, the tertiary centre is a platform for education, agreement on a hospital-adjusted guideline and participation in clinical trials for rare patients, e.g., to describe the natural history and risk for disease progression in patients with iHE and in trials with targeted treatments in iHES [20].

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