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Low Incidence of Anti-PF4/Heparin Antibodies in Patients with Acute Myelogenous Leukemia

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To the editor.

Recent years have seen an increasing interest in antibodies against platelet factor 4 in thrombotic complications. Hence, we want to explore the prevalence of anti-PF4/heparin antibodies in newly diagnosed acute myelogenous leukaemia (AML) patients. In the present study, we demonstrated that the existence of PF4/heparin antibodies is a rare occurrence at the time of diagnosis in AML patients.

AML is an aggressive and highly malignant haematological disease characterized by bone marrow infiltration of immature leukemic blasts suppressing normal haematopoiesis, leading to severe cytopenia.[1] The disease is associated with a high degree of mortality and morbidity, and among commonly occurring complications, we find both bleeding and thrombosis. The pathophysiology behind the haemostatic complications occurring in AML is complex and multifactorial and includes bone marrow failure with severe thrombocytopenia. Also, endothelial dysfunction, inflammation with cytokine activation and coagulopathy with disseminated intravascular coagulopathy (DIC) contribute to these complications.[2] The most studied example is the coagulopathy associated with acute promyelocytic leukemia (APL),[3] although also in other AML cases haemostatic complications are of concern.[2]

Heparin-induced thrombocytopenia (HIT) is a rare condition associated with antibodies against platelet factor 4 in a complex with heparin, which activate platelets, leading to thrombocytopenia accompanied by a pronounced thrombotic state.[4] Both classical HIT and autoimmune HIT variants like delayed-onset- and refractory or persistent HIT are observed after exposure to heparins. However, other HIT-like conditions can, in rare cases, occur in the absence of recent heparin exposure such as spontaneous HIT,[5] or vaccine-induced immune thrombotic thrombocytopenia (VITT) following COVID-19 vaccines,[6,7] and in rare cases after other vaccines,[8] adenovirus infection,[9,10] and monoclonal gammopathy of clinical significance.[11] A relatively high number of anti-PF4/heparin antibody-positive patients have been observed among patients with myeloproliferative neoplasms (MPNs).[12] Based on these considerations, we wanted to examine the presence of anti-PF4/heparin antibody-positive patients in a cohort of newly diagnosed AML patients.

The collection and use of samples for this study were approved by the regional committees for medical and health research ethics (REK) both for biobanking and *in vitro* experimental research (REK Vest 1750/2015 and REK Nord 480847/2022). Registration of collected samples was also approved by the Norwegian Data Protection Authority (reference 02/1118-5). Serum samples from 136 AML patients at the time of diagnosis and before initiating treatment **(Table 1)** were collected and aliquoted before being frozen and stored at -80°C. Antibodies to PF4/heparin were tested for by enzyme-linked immunosorbent assay (ELISA) using LIFECODES PF4 IgG (Immucor), dilution 1:50, with an optical density cutoff value ≥0.400.

Only one of the 136 patients (0.7 %) had a positive test for anti-PF4/heparin antibodies: OD value 0.959, and in the other 135 samples, no anti-PF4/heparin antibodies were detected **(Figure 1).** The ELISA-positive patient was a male patient of 49 years with a diagnosis of AML FAB M4, karyotype, 45; X, -Y, and with the *NPM1* mutation without the *FLT3* mutations. Medical records indicate no previous exposure to heparins. Coagulation parameters at the time of diagnosis demonstrated prothrombin time- INR 1.1 (normal range 0.9-1.2), activated partial thromboplastin time (APTT) 38 seconds (30-44), fibrinogen 4.6 g/L (1.9-4.0), and D-dimer 2.33 mg/L (<0.50). He received induction therapy with the 3+7 regimen, with additional consolidation with cytarabine, and importantly, he had no bleeding or thrombotic complications during the treatment period.

For further analysis of the ELISA-positive patient, the AcuStar HIT-IgG test (Instrumentation Laboratory) with cutoff value ≥ 1.0 U/ml and the Heparin-induced

Table 1. Demographic and clinical data for the 136 AML patients were included in the study.

Characteristics		
Gender (n, %)	Females/males	51 (37.5%) / 85 (62.5%)
Age (years median-range)	66	18-95
-de novo	100	74%
-secondary	33	23%
-relapse	3	2%

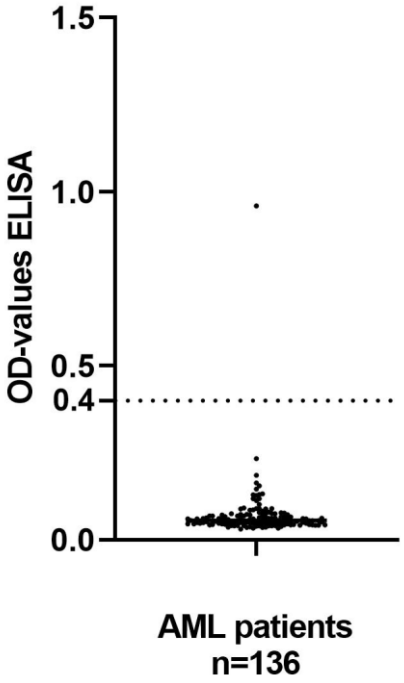

Figure 1. Occurrence of anti-PF4 antibodies in 136 patients with newly diagnosed AML.

multiple electrode aggregometry (HIMEA) run on the multiple analyzers (Dynabyte Medical), as described in Schultz et al,[7] and a PF4-dependent P-selectin expression assay, modified from Samuelson Bannow et al.,[13] were used as functional platelet activation assays. For the ELISA-positive patient, the AcuStar HIT-IgG test and the two functional tests were all negative. In the present study, we demonstrated that the existence of PF4/heparin antibodies is a rare occurrence in AML patients at the time of diagnosis, with an estimated occurrence < 1%, an incidence lower than the existence of such antibodies in a normal population.[14] Hence, the contribution of anti-PF4/heparin antibodies in the coagulopathy seen in the acute phase of AML patients is very low. It should be emphasized that the presence of anti-PF4/heparin antibodies without functional platelet assay has scarce significance. Functional assay should always be performed with the suspicion of PF4-mediated thrombotic conditions. Furthermore, a significant number of AML patients develop thrombosis, including catheter-associated thrombosis (CAT),[15] during their treatment period, and hence often are exposed to heparins.[15] If these patients are more vulnerable to developing anti-PF4/heparin antibodies later in the treatment course and eventually HIT, it remains unanswered. Physicians treating acute leukaemia patients should be reassured by the low occurrence of these antibodies in this study. However, be aware of the potential association after exposure to heparins and other triggers, and the low antibody occurrence does not exclude the possibility of later development of HIT or anti-PF4-driven disease.

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