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The limitation of genetic testing in diagnosing patients suspected for congenital platelet defects

To the Editor:

Congenital platelet defects (CPD) are rare disorders of primary hemostasis caused by congenital defects in platelet production or function. Identification of CPDs is challenging due to the lack of awareness resulting in late or missing referrals, the lack of diagnostic criteria, absence or limitations of laboratory tests and poor standardization of the available tests.[1] However, an accurate diagnosis is important for proper counseling and management of patients and to avoid ineffective and potentially harmful treatments due to misdiagnosis, like idiopathic thrombocytopenic purpura (ITP).

DNA-based analysis has become increasingly important for diagnosing CPDs.[2] Genetic analysis can be useful to confirm a suspected phenotypic diagnosis, and to identify patients with an increased risk for associated pathologies, such as myelofibrosis (*NBEAL2*), renal insufficiency (*MYH9*) and hematological malignancies (*RUNX1*). The International Society for Thrombosis and Hemostasis (ISTH) currently recommends to perform genetic analysis as a third-line investigation, that is, after extensive phenotyping and functional analyses have confirmed the presence of a platelet disorder.[3] Recent studies on the efficacy of genetic testing in selected patients with platelet disorders have suggested that genetic analysis could be moved "upward" in the diagnostic approach in order to simplify and hasten the diagnosis of CPDs.[4-6] However, it remains unclear whether genetic analysis should be performed as a first-line investigation, alongside initial functional analysis of platelet function in unselected patients in whom a congenital platelet disorder is suspected.

In the Thrombocytopathy in the Netherlands (TiN) study, we assessed the diagnostic value of genetic analysis performed in parallel with routine laboratory tests in a prospective cohort of patients suspected of having a CPD. Three categories of patients were included in the study: (a) patients suspected of having a CPD based on previous abnormal platelet counts, light transmission aggregometry (LTA) results or platelet ADP content without a molecular diagnosis (n = 96) (b) patients suspected of having a CPD based on a predominantly mucocutaneous bleeding tendency compatible with a CPD, in whom other known causes of bleeding were excluded and in whom previous LTA results were normal (n = 39), and (c) patients suspected of having a CPD based on a predominantly mucocutaneous bleeding tendency compatible with a CPD, in whom other known causes of bleeding were excluded, newly referred for platelet function testing (n = 21). Laboratory tests were performed for platelet count, aggregation response to four agonists, nucleotide content, surface receptor expression with flow cytometry and whole-exome sequencing (WES) with a selected 76 gene panel (Table S1). A CPD was diagnosed when an abnormal platelet count or function was found on at least two separate occasions, of which one was in our diagnostic laboratory. A possible CPD was diagnosed when an abnormal platelet function was found once in our diagnostic laboratory, or when abnormal platelet function test results were inconsistent with previous findings. In line with the American College of Medical Genetics guidelines, a genetic variant was stated to be causal when a (likely) pathogenic variant (class 4 or 5, respectively)[7] was identified in one or more of the selected genes that corresponded to the platelet phenotype.

In patients with previously abnormal laboratory results, a CPD was confirmed in 61 of 96 (64%) patients, and a possible CPD was diagnosed in four of 96 (4%) patients. Eight of 96 (8%) patients received a molecular diagnosis, and in 11 of 96 (11%) patients a variant of unknown significance was identified (Table 1). In patients with previously normal LTA results and in newly referred patients, a possible CPD was diagnosed in 10 of 39 (26%) and six of 21 (29%) patients, respectively. No causal genetic variants were identified in these patients.

We included several subgroups of patients suspected of having a CPD to properly assess when genetic analysis should be performed in the diagnostic procedure. Our study shows that the diagnostic yield of genetic analysis is limited in patients suspected for a CPD, since only 5% (8/156) of patients received a molecular diagnosis. This is in contrast to the diagnostic rate of 47.8% for platelet count defects, and 26.1% for platelet function defects reported in a recent study. There, 2396 patients with bleeding, thrombotic, and platelet disorders (BTPD) were screened with a panel of 96 BTPD-associated genes, in which the number of platelet associated genes was similar to our gene panel.[8] However, their diagnostic rate included variants of unknown significance, resulting in an overestimation. Leaving out variants of unknown significance strongly reduced the diagnostic rate. The differences between their and our study are also related to patient-selection. Our study reflects the real-life population of patients suspected for a CPD referred to outpatient clinics of hemophilia treatment centers Their study included patients with a previously ascertained pathogenic variant, or patients with phenotypes strongly indicative of a particular disorder on the basis of laboratory abnormalities, with a high likelihood of having an inherited BTPD. In patients with either normal laboratory assays or assays not diagnostic of an established disorder, they reported a diagnostic rate of only 3.2%. Studies performed in the Iberian Peninsula, with a gene panel similar to ours, reported the identification of a molecular defect in 40%[9] and 68%[5] of patients with (suspected) CPDs. Their studies included large numbers of patients with Glanzmann thrombasthenia,

TABLE 1 Results of laboratory and genetic testing per patient category

Patient category	N	CPD	Possible CPD	Molecular diagnosis	VUS
Previously abnormal laboratory tests[a]	96	61 (64)	4 (4)	8 (8)	11 (11)
Previously normal LTA results[b]	39	0 (0)	10 (26)	0 (0)	1 (3)
Newly referred[c]	21	0 (0)	6 (29)	0 (0)	1 (5)

Note: Data are presented in number of patients (%).
Abbreviations: CPD, congenital platelet defect; LTA, light transmission aggregometry; VUS, variants of uncertain significance.
[a]Patients suspected for a CPD based on previous abnormal platelet counts, LTA results or platelet ADP content without a molecular diagnosis.
[b]Patients suspected for a CPD based on a predominantly mucocutaneous bleeding tendency compatible with a CPD, in whom other known causes of bleeding were excluded and in whom previous LTA results were normal.
[c]Patients suspected for a CPD based on a predominantly mucocutaneous bleeding tendency compatible with a CPD, in whom other known causes of bleeding were excluded, newly referred for platelet function testing.

Bernard-Soulier syndrome and MYH9-related disorders. Therefore, their study population does not reflect clinical practice and is not comparable to ours. A study performed in a pediatric population reported a positive molecular diagnosis in 23.8%.[10] Their cohort included a relatively large number of patients with thrombocytopenia (67% vs 22% in our cohort) and genetic testing was not performed as a first-line investigation.

It is possible that limitations of WES have led to an underestimation of the number of patients with an identified genetic variant. First, large insertions and deletions might be missed. Second, regulatory and non-coding regions of the genome were not examined, and these regions might harbor variants essential for controlling transcriptional regulation or splicing. Third, by using a selected gene panel we might have missed pathogenic variants in genes not included in the panel. Finally, we cannot exclude that an additive effect of multiple genetic variants that have escaped our selection, might underlie the CPD in individual patients.

In conclusion, genetic testing with a selected gene panel has limited diagnostic yield in patients suspected for a CPD and should only be performed in patients in whom a platelet number or function defect is confirmed.

CONFLICT OF INTEREST

The authors state that they have no conflict of interest.

ORCID

Maaike W. Blaauwgeers https://orcid.org/0000-0002-6254-5335
Albert Huisman https://orcid.org/0000-0002-2291-2487
Rolf T. Urbanus https://orcid.org/0000-0002-1601-9393

Maaike W. Blaauwgeers, Ivar van Asten, Marieke J.H.A. Kruip, Erik A.M. Beckers, Michiel Coppens, Jeroen Eikenboom, Karin P.M. van Galen, Albert Huisman, Suzanne J.A. Korporaal, Hans Kristian Ploos van Amstel, Rienk Y.J. Tamminga, Rolf T. Urbanus, Roger E.G. Schutgens

[1]*Van Creveldkliniek, University Medical Center Utrecht, University Utrecht, Utrecht, The Netherlands*
[2]*Van Creveld Laboratory, University Medical Center Utrecht, University Utrecht, Utrecht, The Netherlands*
[3]*Center for Circulatory Health, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, University Utrecht, Utrecht, The Netherlands*
[4]*Department of Haematology, Erasmus University Medical Center, Rotterdam, The Netherlands*
[5]*Department of Hematology, Maastricht University Medical Center, Maastricht, The Netherlands*
[6]*Department of Vascular Medicine, Amsterdam Cardiovascular Sciences, Amsterdam University Medical Center, location AMC, Amsterdam, The Netherlands*
[7]*Department of Internal Medicine, division of Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, The Netherlands*
[8]*Laboratory of Experimental Cardiology, University Medical Center Utrecht, University Utrecht, Utrecht, The Netherlands*
[9]*Department of Medical Genetics, University Medical Center Utrecht, University Utrecht, Utrecht, The Netherlands*
[10]*Department of Pediatric Hematology, Beatrix Children's Hospital, University Medical Center Groningen, Groningen, The Netherlands*

Correspondence
Roger E.G. Schutgens, Van Creveldkliniek, University Medical Center Utrecht, University Utrecht, Heidelberglaan 100, room C01.414, 3584 CX, Utrecht, The Netherlands.
Email: r.schutgens@umcutrecht.nl

Maaike W. Blaauwgeers and Ivar van Asten contributed equally.

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Axicabtagene ciloleucel for CD19+ plasmablastic lymphoma

To the Editor:

Plasmablastic lymphoma (PBL) is an uncommon, high-grade B cell neoplasm. While recognized by the WHO classification as a subset of large B-cell lymphoma, its plasma cell-like immunophenotype, morphology, and association with HIV and EBV reactivation distinguish PBL from typical diffuse B-cell lymphoma.[1] Current guidelines suggest initial therapy with dose-adjusted infusional etoposide, vincristine and doxorubicin with bolus cyclophosphamide and prednisone (DA-EPOCH), reflecting its classification as a large B-cell lymphoma.[2] Median overall survival with this approach is disappointing, reported as 15 months in HIV-positive patients, and likely worse in HIV-negative patients.[3,4]

PBL is challenging to differentiate from plasma cell myeloma, and several reports have suggested improved survival with addition of anti-myeloma agents such as bortezomib and lenalidomide.[5] Despite these advances, PBL remains a highly aggressive malignancy and new approaches are necessary.

We report here a case of relapsed/refractory CD19+ PBL treated with axicabtagene ciloleucel (axi-cel), a CD19-directed chimeric antigen receptor (CAR) T cell therapy approved by the FDA for large B-cell lymphoma that is relapsed or refractory after two or more lines of systemic therapy.

Our patient was a previously healthy 31-year-old female who presented with fatigue and back pain. Initial laboratory evaluation was remarkable for a hemoglobin of 5 g/dL and serum calcium of 18 mg/dL. A CT scan demonstrated a pulmonary embolism, multiple pathologic rib fractures, widespread bony lucencies, diffusely enlarged kidneys, and bilateral, large pleural effusions. Pleural fluid cytology revealed a malignant B lymphoid neoplasm with plasmacytic features. HIV serology and EBV in situ hybridization were negative.

A bone marrow biopsy demonstrated 100% replacement by large cells (Figure 1A). The Ki67+ fraction approached 100%. On flow cytometry, the cells were lambda-restricted, expressing CD10, CD19, CD38 (bright), CD45(dim),CD138 (dim), and negative for CD5, CD20, CD23, CD34, and CD56 (Figure 1B, left). Fluorescent in situ hybridization identified gain of 1q21 and was negative for MYC re-arrangement. Her serum protein electrophoresis was negative for a monoclonal spike, and her serum free lambda and kappa light chains were 133 and 20 mg/L respectively.

She was diagnosed with PBL and began therapy with DA-EPOCH. After two cycles she was switched to ifosfamide, carboplatin, and etoposide (ICE) due to progressive disease. PET/CT performed after her first cycle of ICE demonstrated widespread disease, with nearly confluent fluorodeoxyglucose (FDG) uptake throughout her skeleton and kidneys (Figure 1C). A lumbar puncture showed no disease involvement of her central nervous system by cytology and flow cytometry.

She began salvage treatment with carfilzomib, lenalidomide, daratumumab, and dexamethasone. Her symptoms and lambda FLCs level improved, but her cytopenias persisted and repeat bone marrow biopsy performed 2 months after initiation of this regimen demonstrated 70% involvement of her PBL and reconfirmed CD19 expression.

The patient underwent autologous leukapheresis for axi-cel manufacturing while continuing her prior regimen during the manufacturing process. About 6 months from her initial presentation, she received cyclophosphamide and fludarabine for lymphodepletion followed by axi-cel. After axi-cel infusion, she developed grade 2 cytokine release syndrome with high fevers, a peak ferritin of 18 330 ng/mL and grade 2 neurologic toxicity (grade determined based on ASTCT consensus scheme). She did not receive tocilizumab or steroids. At her 6 week follow-up, her cytopenias had resolved, her lambda FLCs had decreased from a pre-treatment level of 91.3 to <1.5 mg/L, and her pain had significantly improved. At 4 months after axi-cel, a PET/CT showed a complete metabolic response (Figure 1C). Unfortunately, a bone marrow biopsy performed 5 months post-treatment to assess depth of response found 50% involvement by residual/recurrent CD19+ PBL.

One month later, at 6 months post-treatment, the patient developed severe thrombocytopenia and spontaneous tumor lysis; she was found to have circulating PBL cells and elevated lambda FLC at 142.1 mg/L. She was admitted for stabilization and initiation of salvage blinatumomab, which was chosen given her preserved CD19 expression (Figure 1B). She tolerated blinatumomab well but did not show any evidence of immediate response. Shortly after discharge