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POSIZIONE RICOPERTA DIRIGENTE MEDICO

ESPERIENZA PROFESSIONALE

Da Giugno 2024 DIRIGENTE

Ospedale Maggiore Policlinico

Dirigente Medico (Patologia Clinica e Biochimica Clinica) presso SC Patologia Clinica dell'Ospedale Maggiore Policlinico

Attività o Settore SANITA'

Da Settembre 2023 a Maggio 2024 DIRIGENTE

Ospedale Maggiore Policlinico

Dirigente Medico presso il Centro Regionale di Riferimento Lombardia/Nord Italia Transplant program (NITp), Unità Operativa Complessa Coordinamento Trapianti dell'Ospedale Maggiore Policlinico

Attività o Settore SANITA'

ISTRUZIONE E FORMAZIONE

Da Gennaio 2021 a Febbraio 2025 Specializzazione in Patologia Clinica e Biochimica Clinica (80/80)

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Università di Pisa (laurea conseguita all'estero con successivo riconoscimento di equipollenza in Italia)

Altre lingue

	COMPRENSIONE		PARLATO		PRODUZIONE SCRITTA
	Ascolto	Lettura	Interazione	Produzione orale	
Italiano	C2	C2	C2	C2	C2
Inglese	B1	B2	B1	B1	B1

Livelli: A 1/2 Livello Base - B 1/2 Livello Intermedio - C 1/2 Livello Avanzato

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Patente di guida B

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Autorizzo il trattamento dei miei dati personali ai sensi del Decreto Legislativo 30 giugno 2003, n, 196 'Codice in materia di protezione dei dati personali'.

FIC Federazione Italiana di Cardiologia
Italian Federation of Cardiology

Annular enlargement for failed aortic root homograft

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J Cardiovasc Med 2012, 13:413–414

Keywords: aortic homograft, aortic valve replacement, enlargement of the aortic annulus

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To the Editor

Replacement of an aortic homograft used for aortic root reconstruction is a challenging procedure.[1,2] We report a case of a patient in whom replacement of an aortic homograft valve required an annular enlargement procedure due to a residual diminutive aortic annulus.

Methods and results

A 33-year-old man was admitted to our department for correction of severe aortic valve incompetence. At the age of 6 months, he had undergone repair of aortic coarctation and 9 years previously, because of endocarditis on a bicuspid aortic valve, full root replacement using a cryopreserved homograft was done using multiple single stitches. On admission, he was in sinus rhythm with an enlarged cardiac shadow on chest radiograph. Transthoracic two-dimensional echo showed a 4+ aortic regurgitation, a dilated left ventricular cavity with an end-diastolic volume of 206 ml and an ejection fraction of 36%, and a pulmonary artery pressure of 60 mmHg. Angiography confirmed the severe aortic incompetence and showed normal coronary ostia and arteries. At operation, the chest was re-entered through a repeat median sternotomy. Moderately hypothermic cardiopulmonary bypass was instituted by cannulation of the ascending aorta and right atrium, whereas myocardial protection was achieved with cold Custodiol cardioplegia into the coronary ostia and by topical cooling. The homograft aortic root, which appeared thickened but not calcified, was opened just below the previous suture line. The aortic cusps were retracted and fibrotic with mild annular calcification. Excision of the homograft valve and annular debridement left a rigid and diminutive aortic annulus (20 mm). Anticipating a potential prosthesis–patient mismatch, it was decided to enlarge the aortic annulus using a previously described technique.[3,4] The aortotomy was extended in the commissure between the left and noncoronary sinuses into the underlying interleaflet triangle taking care to avoid opening of the left atrial roof. The resulting defect of the aortic wall was closed with a patch of bovine pericardium using a continuous suture of 4/0 polypropylene (Fig. 1). A 23-mm Sorin Bicarbon Slimline prosthesis (Sorin Biomedica Cardio, Saluggia, Italy) was inserted using multiple sutures reinforced by subannular Teflon pledgets; at the level of the pericardial patch, stitches were passed from outside to inside. The pericardial patch was then trimmed and partly used to close the aortotomy. The patient recovered uneventfully and was discharged on the seventh postoperative day. A control two-dimensional echo showed a peak transprosthetic gradient of 19 mmHg, with a calculated effective orifice area index (EOAI) of 1.23 cm^2/m^2, and a left ventricular ejection fraction of 34% with a reduction of pulmonary artery pressure (38 mmHg).

Comment

Reoperation in patients with a failing aortic homograft implanted as total root is associated with a relatively high perioperative morbidity.[1] Surgical strategies usually depend on the degree of calcification of the aortic wall; in the case of extensive calcification, '*en bloc*' homograft removal is necessary, whereas in the presence of a relatively pliable, noncalcific homograft wall, isolated aortic valve replacement may be performed as in the present case. However, in our patient, excision of the aortic cusps left a small and rigid annulus not fitting a 21-mm mechanical prosthesis, an option that was preferred because of his young age and previous operations. Furthermore, with a 21-mm prosthesis, the expected EOAI was 0.83 cm^2/m^2 anticipating a moderate prosthesis–patient mismatch according to Mohty *et al.*.[5] Thus, insertion of a larger prosthesis was felt indicated and this was achieved by enlarging the aortic annulus with the technique described above. This quite simple technique, proposed by Nunez *et al.*[6] in 1983 and later employed by us with gratifying results,[3,4] proved feasible and effective also when enlargement of the aortic annulus in replacing a failing aortic valve in a homograft root is required. This technique, which is not mentioned even in large series of aortic allograft reoperations,[2] is, however, possible only in the absence of coarse calcification of the homograft root; if calcification is present, root replacement appears mandatory.[1] Finally, predischarge two-dimensional echocardiographic control showed an adequate EOAI, thus eliminating the hazard of a prosthesis–patient mismatch,

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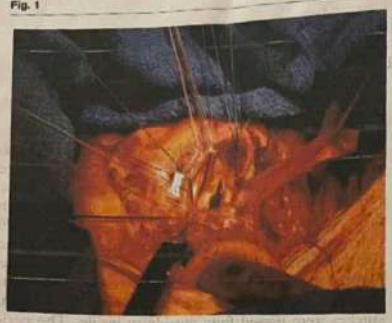
Fig. 1



Intraoperative view showing insertion of the pericardial patch used to enlarge the aortic annulus.

particularly deleterious in patients with depressed left ventricular function.[4,7–12]

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Preziose informazioni dall'esame del liquido pericardico

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ABSTRACT

Valuable information from the examination of the pericardial fluid.
A 63-year-old man, without previously known cancer disease, arrived at the Emergency room because of severe dyspnea. Electrocardiogram revealed right branch block and low ventricular repolarization voltages. Chest X ray and echocardiogram, instead, showed right pleural effusion and cardiac massive effusion. The patient underwent pericardiocentesis. The morphological study of pericardial fluid revealed the presence of numerous nonhematopoietic cells and the patient was admitted to the hosptital Thoracentesis was performed during hospitalization. The analysis of pleural fluid showed similar results to those obtained in pericardial fluid. A subsequent paracentesis was performed. Accordingly, this fluid showed similar atypical cells. Adenopathy in the supraclavicular area was found at the TC scan. Lymphnode biopsy confirmed a poorly differentiated proliferation, consistent with squamous cell carcinoma. In conclusion, morphological analysis of the pericardial fluid is a simple examination that, in a short time, may alert about the presence of unexpected atypical cells.

Parole chiave: *liquido pericardico, esame microscopico, citocentrifuga*

CASO CLINICO

Un uomo di 63 anni, fumatore, arriva in pronto soccorso, con sintomi di dispnea ingravescente da due giorni. Il paziente soffre di diabete mellito di tipo 2, per cui è in terapia con ipoglicemizzante orale, e presenta una ipertensione arteriosa in terapia con sartani. L'anamnesi per neoplasie è muta. All'arrivo il paziente è marcatamente tachipnoico (22 atti respiratori/minuto), dispnoico e tachicardico. All'auscultazione si rilevano murmure vescicolare abolito alla base polmonare destra e toni cardiaci parafonici. L'elettrocardiogramma mostra fibrillazione atriale (130 battiti/minuto), blocco di branca destra e bassi voltaggi della ripolarizzazione ventricolare. La radiografia del torace evidenzia un'ombra cardiaca ingrandita e un versamento pleurico di destra.

L'ecocardiogramma mostra un tamponamento cardiaco, quindi, in regime di urgenza, il paziente viene sottoposto a pericardiocentesi e vengono inviati i campioni per gli esami chimico-fisico, morfologico e colturale in laboratorio. Il liquido pericardico (LP), al conteggio con microscopio ottico (camera di Burker), presenta 6,8 x 10^9 leucociti/L. Questo valore viene confermato anche all'analisi strumentale (XN-1000, modulo *"body fluid"*, Sysmex Corporation, Kobe, Japan). In più, il grafico strumentale evidenzia la presenza di cellule di natura sospetta, localizzate nella parte alta del grafico "WBC differential fluorescence (WDF)" e quindi dotate di grandi dimensioni e di elevata fluorescenza. Con l'analisi morfologica, dopo cito-centrifugazione (Cytospin 4, ThermoScientific + colorazione con May Graunwald-Giemsa), si visualizzano un tappeto di emazie, granulociti

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neutrofili (11%), linfociti (70%), elementi monocito-macrofagici (19%) e numerose cellule di natura non emopoietica con aspetto dismorfico (Figura 1). Il risultato viene pertanto comunicato al pronto soccorso

Il paziente viene trasferito in reparto e, durante la degenza, per la presenza di versamento pleurico, viene eseguita una toracentesi. Un campione del liquido prelevato viene inviato in laboratorio. L'analisi del liquido pleurico, condotta con una procedura analoga al LP, mostra numerose emazie, granulociti neutrofili (3%), linfociti (45%), elementi monocito-macrofagici (52%) e nuovamente numerosi cluster di cellule sospette per neoplasia, simili a quelle trovate nel LP e illustrate in Figura 1. Alcune cellule, con caratteristiche morfologiche simili, si trovano anche nel liquido ascitico, prelevato per presenza di versamento addominale.

L'analisi citopatologica dei liquidi conferma il sospetto di neoplasia. L'aspirato pericardico, osservato al microscopio dopo colorazione con ematossilina-eosina (ingrandimento 400x), evidenzia la presenza di abbondante detrito ematico con alcuni granulociti e isolati elementi atipici di grande taglia, sospetti per neoplasia. I marcatori tumorali sierici valutati (CA 19-9, CEA, antigene prostatico e alfa-fetoproteina) non presentano valori significativamente alterati. La valutazione microbiologica del liquido è negativa. Alla tomografia (TC) del torace si osservano una trombosi a livello dell'arteria polmonare comune e adenomegalie multiple, in sede paratracheale e sovraclaveare. Viene effettuata pertanto un'agobiopsia del linfonodo sovraclaveare che conferma la presenza di una proliferazione scarsamente differenziata, la cui natura epiteliale e la differenziazione squamocellulare risultano confermate con le immunocolorazioni per citocheratine 5/6 e p40 (Figura 2).

Il paziente, dopo un mese di ospedalizzazione, decede a causa di insufficienza respiratoria e renale.

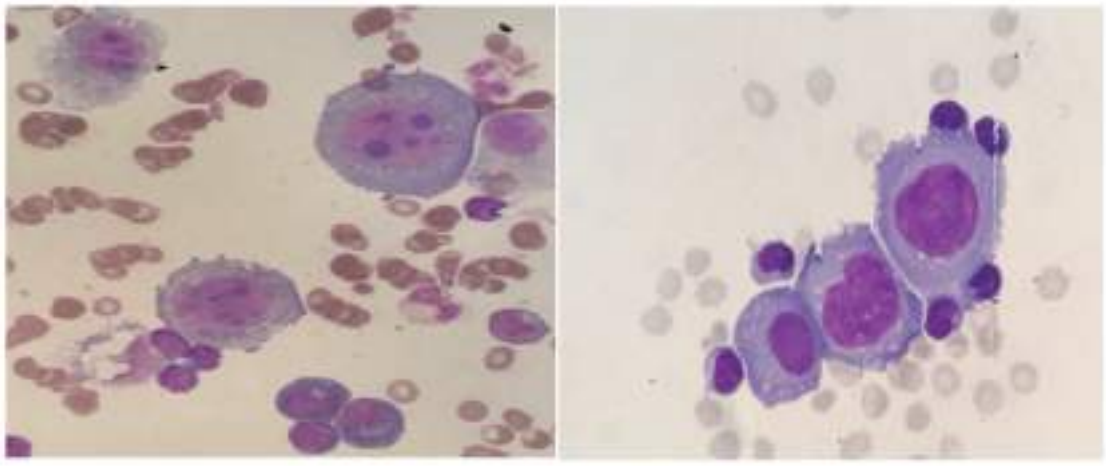

Figura 1
Liquido pericardico (a sinistra) e liquido pleurico (a destra) osservati al microscopio ottico (ingrandimento 600x), dopo aver sottoposto il campione a citocentrifugazione e colorazione con May-Grunwald Giemsa.

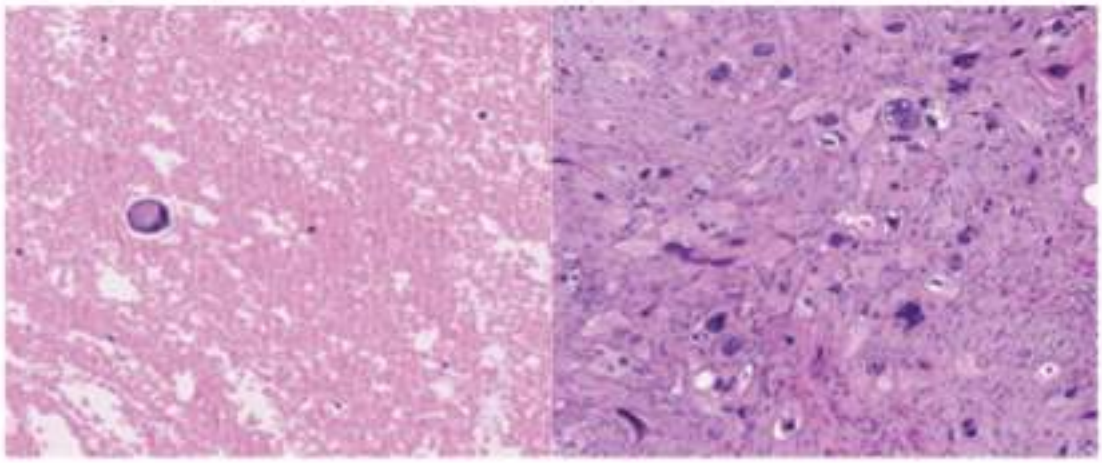

Figura 2
Aspirato pericardico (a sinistra) e biopsia linfonodale (a destra) osservati al microscopio ottico (ingrandimento 400x), dopo colorazione con ematossilina-eosina.

DISCUSSIONE

Il pericardio, una sottile membrana di origine mesodermica, è un sacco fibro-sieroso che contiene il cuore e il tratto iniziale dei grossi vasi. Questa struttura è costituita da due strati distinti: pericardio fibroso e sieroso. Il LP, che si trova in piccole quantità (20-60 mL) tra i due strati del pericardio, è prodotto dalle cellule mesoteliali delle membrane e funge da lubrificante per i movimenti del cuore (1). Varie condizioni e patologie possono causare infiammazione del pericardio (pericardite) e/o eccessivo accumulo del LP (versamento pericardico): infezioni , neoplasie, malattie del tessuto connettivo (2,3). In circa un terzo dei casi, il versamento pericardico è associato a farmaci chemioterapici, radioterapia, alterato drenaggio linfatico dovuto a linfadenopatia mediastinica o pericardite infettiva. Il versamento pericardico maligno è una manifestazione comune e grave nelle neoplasie maligne (4). La lesione primaria più comune per un versamento pericardico maligno è il tumore polmonare. In alcuni casi il coinvolgimento pericardico è la prima presentazione clinica della malattia neoplastica (5). Lo sviluppo del versamento è insidioso (6) e quasi i due terzi di questi pazienti non presentano sintomi e segni cardiovascolari (7). Il versamento aumenta la pressione intra-pericardica in modo graduale, con conseguente diminuzione diastolica e del riempimento cardiaco. Dispnea, tachicardia, ipotensione e pressione venosa giugulare elevata indicano una compromissione emodinamica. Il tamponamento cardiaco è una forma grave di versamento pericardico che rappresenta un'emergenza medica. Un esame obiettivo accurato e un'ecocardiografia tempestiva confermano la presenza di versamento pericardico o tamponamento cardiaco. La pericardiocentesi è la rimozione del LP in eccesso mediante aspirazione, un'operazione necessaria per la decompressione del miocardio (8). La prognosi del paziente dipende dalla causa del tamponamento cardiaco e dalla velocità di adozione di un trattamento appropriato. Le valutazioni citologiche del LP vengono utilizzate per determinare l'eziologia di un versamento pericardico e in particolare per valutare la presenza di una possibile forma tumorale, mentre gli esami microbiologici in laboratorio possono aiutare a identificare un'eventuale infezione (2).

Il caso in questione si riferisce ad un paziente maschio con anamnesi muta per neoplasia, che si è presentato al pronto soccorso per dispnea. In seguito alla rilevazione ecocardiografica di tamponamento cardiaco, il liquido prelevato con pericardiocentesi è stato inviato in laboratorio. L'analisi delle cellule presenti (strumentale e al microscopio ottico) ha rilevato la presenza di cellule di natura sospetta per neoplasia. Durante il ricovero, il paziente ha sviluppato ulteriori versamenti in sedi pleurica e peritoneale; l'analisi citologica di entrambi i liquidi raccolti ha mostrato che le cellule avevano le stesse caratteristiche morfologiche. La TC ha rilevato molteplici adenomegalie; la successiva biopsia linfonodale ha evidenziato una presenza indicativa, per la natura epiteliale e per la differenziazione squamocellulare, di una proliferazione scarsamente differenziata. I tumori a partenza sconosciuta, o tumori epiteliali primitivi occulti, si manifestano nella maggior parte dei casi con metastasi a distanza, per le quali non è definita la sede di origine anatomica primaria. Tali neoplasie hanno manifestazioni cliniche varie e una prognosi infausta nella maggior parte dei casi. Il carcinoma squamoso primitivo occulto rappresenta il 5% di tutti questi tumori e può avere sedi metastatiche a livello dei linfonodi sovraclaveari, come nel caso clinico presentato (9). La scelta della terapia si basa sulle condizioni generali del paziente e sulla probabilità di una risposta a lungo termine al trattamento della malattia neoplastica sistemica. Il versamento pericardico, dovuto all'invasione del tumore o al trattamento del tumore stesso, è uno dei risultati incidentali più comuni nei pazienti oncologici, che peggiora significativamente la morbilità e la mortalità. Gli studi sugli esiti clinici del versamento pericardico maligno hanno riportato un tasso di sopravvivenza a un anno del 10-27% e una sopravvivenza media da 5,4 a 8 mesi. L'attento esame citomorfologico degli aspirati del liquido pericardico è un metodo prezioso, affidabile e utile alla diagnosi (10). L'osservazione di un liquido al microscopio ottico, conoscendo la morfologia delle cellule emopoietiche e riconoscendo gli elementi atipici, può permettere, tramite segnalazione immediata al reparto, di condurre approfondimenti diagnostici indirizzati verso una diagnosi corretta e con una tempistica ottimale per quelle patologie che hanno uno sviluppo rapido. In questo caso clinico è stata sottolineata l'importanza di un'analisi morfologica accurata che, nella sua semplicità e rapidità di esecuzione può dare un'indicazione diagnostica molto significativa ed utile, con un costo molto ridotto.

La malattia pericardica maligna ha un'eziologia complessa con un ampio spettro di presentazione clinica, dal paziente asintomatico a quello con esito potenzialmente letale. È molto importante stabilire l'eziologia del versamento pericardico, per una diagnosi rapida e una terapia mirata. La gestione dei pazienti è pertanto multidisciplinare, all'interno della quale il laboratorio, collaborando attivamente con i clinici di riferimento, gioca un ruolo fondamentale.

CONFLITTO DI INTERESSE

Nessuno

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DEVELOPMENT AND PRELIMINARY EVALUATION OF A METHOD FOR THE DETERMINATION OF GROWTH HORMONE IN NEWBORNS ON DRIED BLOOD SPOTS

Vantaggiato C[1], Giavoli C[2,3], Arosio M[2,3], Giacchetti F[3], Sharif Rouhani A[1], Di Deo P[1], Varani C[1], Modica Amore M[1], Di Modugno A[1], Napolitano F[1], Vidali M[1], Ceriotti F[1]

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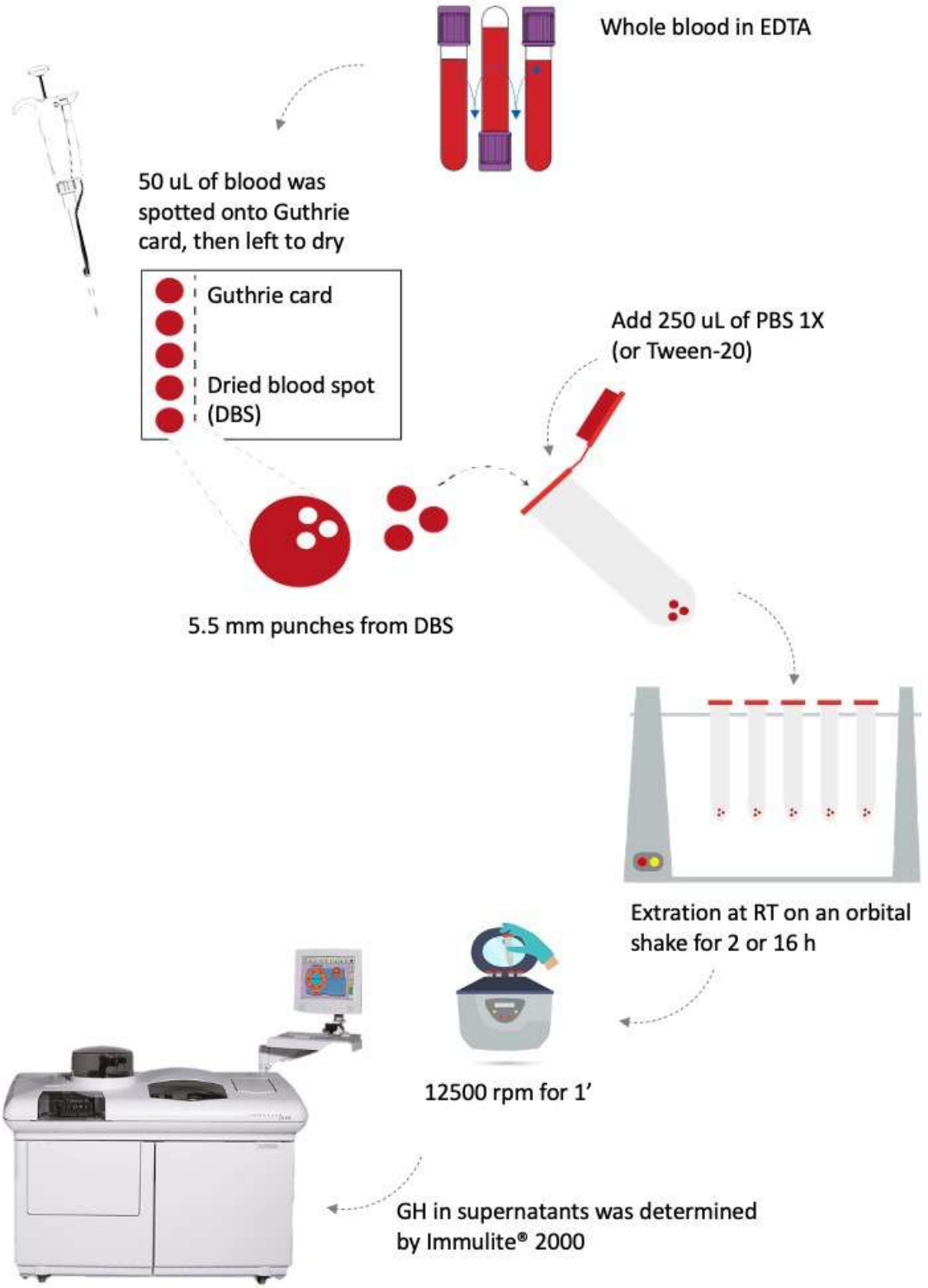
Background and Aim:

- Severe deficiency of GH of the newborn is a rare but potentially life-threatening disease.
- GH can be measured during the first week of life when levels are physiologically higher (neonatal hypersomato-tropism).
- GH evaluation using dried blood spots (DBS) may offer several advantages: easier transportation and storage, reduced costs, allowing centralization and method standardization.

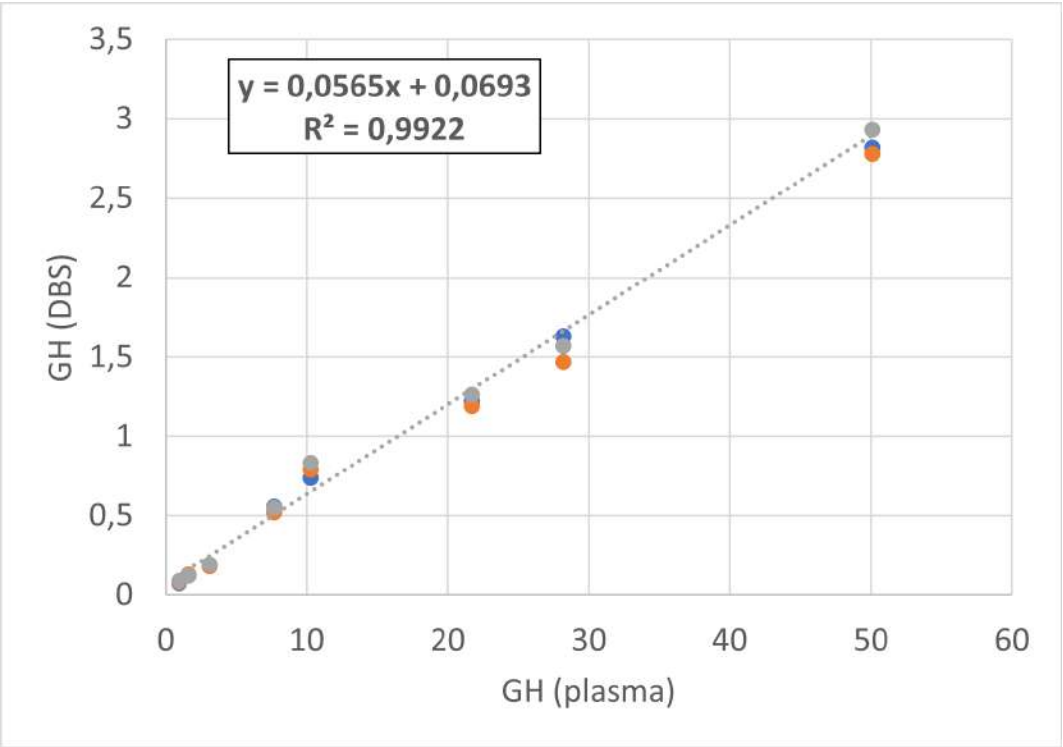
- **Aim** of the study was to validate a method for measuring GH in newborns from DBS.

Blood spot sample preparation



Results 1:

- Interference on Immulite by hemolysis present after extraction was evaluated by spiking extracted samples with known concentrations of GH (2 experiments at 3.2 and 9.0 µg/L). No interference was detected (recovery>99%).
- A calibration curve was built by plotting GH measured in serum vs extracted GH (3 independent replicates, 8 levels from 1 µg/L to 50 µg/L).
- Linearity was verified (R^2>0.99) up to a GH serum concentration of 50 µg/L. Recovery at each level was >90%.



Interference:	No interference by hemolysis
Linearity:	Range 1-50 µg/L (R^2>0.99)
Recovery:	>90%

Results 2:

- Repeatability at the 8 tested concentrations was 11.1%, 2.4%, 3.5%, 3.5%, 6.1%, 2.9%, 5.2%, 2.7%. Further precision experiments (6 independent replicates at 7.7 µg/L) confirmed previous observations (CV%=3.7%).
- No appreciable differences were found between samples stored at -20°C up to 2 months or directly processed (similar serum/DBS ratio and recovery>90%), or between samples extracted with Tween or onlyPBS (at 50 µg/L differences <5%), or between samples incubated for 2 or 16 hours (at 2.9, 7.7 and 50 µg/L %differences were 4.2%, 5.9% and 12.6%).

Repeatability:	from 2.4% (1.6µg/L) to 11.1% (1µg/L) 3.7% at 7.7 µg/L
Stability (-20°C 2mo):	Recovery >90%
Extraction (Tween vs PBS):	Differences <5%
Incubation (2h vs 16 h):	Differences (4.2%, 5.9% and 12.6% at 2.9, 7.7 and 50 µg/L)

Conclusions:

- Preliminary evaluation suggests that this method can be used to measured GH in newborns using DBS.

References:

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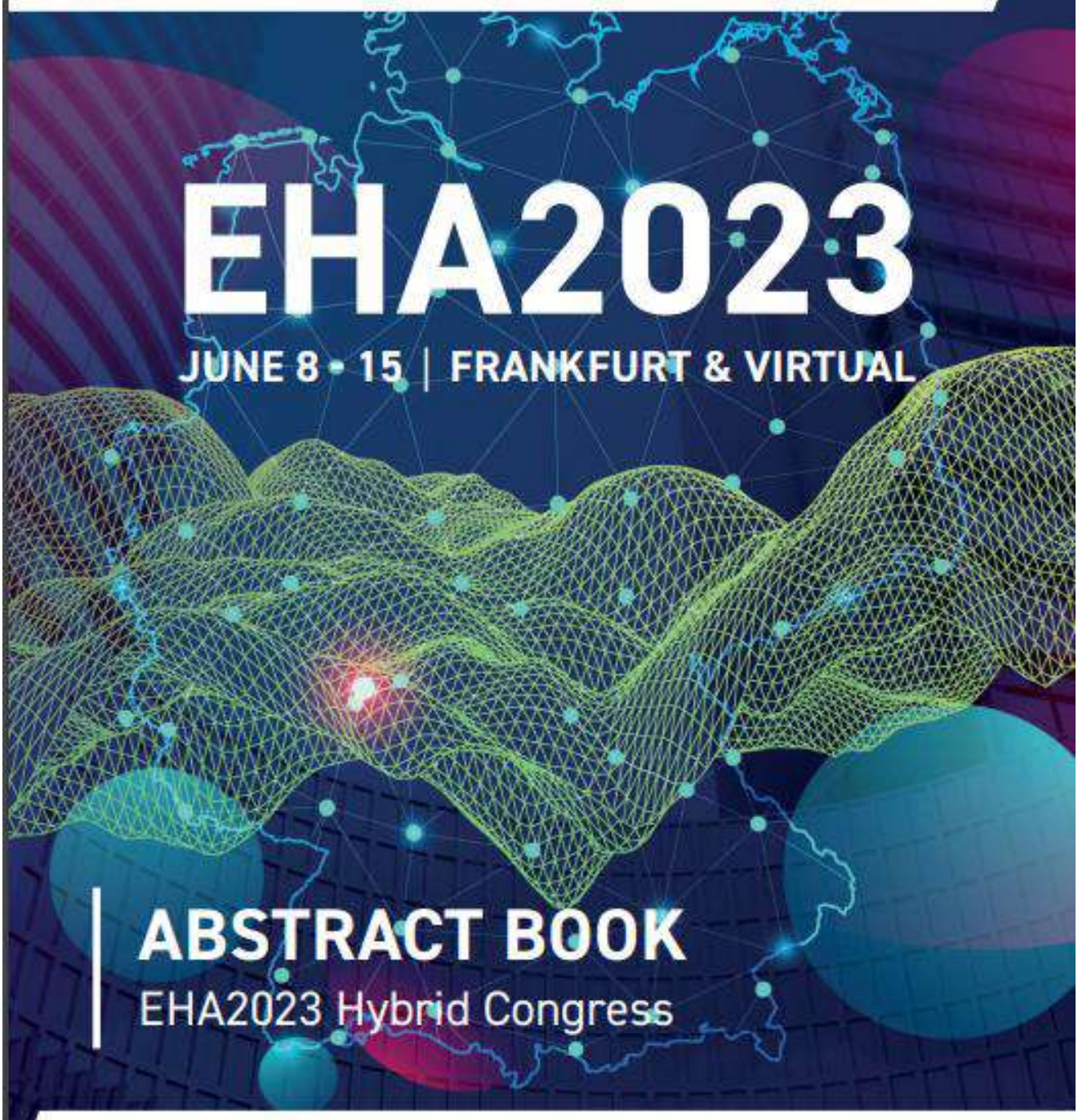

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ABSTRACT BOOK

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PB2546 DIAGNOSTIC POWER OF ERYTHROCYTE AND RETICULOCYTE AUTOMATIC PARAMETERS IN THE SCREENING FOR CONGENITAL HEMOLYTIC ANEMIAS

Topic: 28. Enzymopathies, membranopathies and other anemias

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Background:

Congenital hemolytic anemias (CHAs) are a group of disorders caused by defects of red cell membrane or metabolism, or by abnormal hemoglobin. Due to their rarity and heterogeneity, diagnosis is often challenging, and requires specific tools not always available outside reference centers; as a consequence, many cases may remain undiagnosed or misdiagnosed. The availability of additional parameters for complete blood count has emerged in recent hematology analyzers; however, a few studies have been conducted on advanced RBC parameters and hemolytic anemias. Diagnostic algorithms for screening of hereditary spherocytosis (HS) have been reported using Sysmex XE and Sysmex XN analyzers.

Aims:

We investigated the use of Sysmex parameters, the percentage of microcytes (MicroR) and hypochromic red blood cells (Hypo-He), as well as the immature fraction of reticulocytes (IRF) in combination with complete blood and reticulocyte count, for screening HS and pyruvate kinase deficiency (PKD) with the final aim to develop a diagnostic algorithm for differential diagnosis of CHAs.

Methods:

Complete blood count and reticulocytes from 319 patients with a confirmed diagnosis of CHAs were analyzed (In particular, HS: 81; PKD: 18; autoimmune hemolytic anemia: 50; congenital dyserythropoietic anemia: 9 patients; PIEZO1 gene mutations/ stomatocytosis: 9; thalassemia and sickle cell disease: 118 patients; and the other CHAs: 34 patients) on Sysmex XN analyzer. To further increase number of cases, data were merged with that of 94 other CHAs patients, including 61 HS, available from literature and analyzed on the same instruments (F. Mullier et al. 2011; F. Persijn et al. 2012; V. Bobée et al. 2018; Sottiaux et al. 2020). Patients were stratified according to the severity of anemia (Hb <8g/dL; Hb 8-12g/dL and Hb >12g/dL) and ROC curve analysis was performed for each parameter and condition to establish optimal cut-off limits. A control group of 149 patients with hematological disorders of different etiology, and an extended database of 11.194 (blood count results) cases has been used to establish algorithm disease specificity.

Results:

All 142 HS patients had RET>75x10^9/L and RET/IRF>7.7 ("pre-screening" condition) and were characterized by reticulocytosis without an increase in IRF, increased MicroR without significant increase of Hypo-He. Instead, PKD patients showed increased IRF (>25% and >15,6% in non-anemic and anemic, respectively) accompanied by reticulocytosis, MicroR and Hypo-He reduction. Based on ROC curves analysis results, by combining parameters (Hb, RET, IRF, MicroR, Hypo-He) a diagnostic algorithm was developed for screening of HS and PKD showing a sensitivity and specificity of 97.9% and 92.6% for HS and 94.4% and 99.2% for PKD respectively (figure 1). The

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algorithm showed a superior diagnostic performance when all patients were tested in parallel by using all the previous algorithms reported in literature.

Summary/Conclusion:

The presented algorithm proposes an easy, economic and efficient approach to detect HS and PKD using Sysmex analyzers, definitely contributing to an early diagnosis and a better management of these patients.

Abstract Book Citations: Authors, Title. HemaSphere, 2023;7(S3):pages. The individual abstract DOIs can be found at https://journals.lww.com/hemasphere/pages/default.aspx.

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The 66th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Toward the Creation of Diagnostic Algorithms for Congenital Hemolytic Anemia Using Advanced Parameters from Modern Hematology Analyzers

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Introduction. Congenital hemolytic anemias (CHAs) are a group of disorders caused by defects of red blood cells membrane, metabolism, erythroid maturation, or abnormal hemoglobin. Due to their rarity and heterogeneity diagnosis is challenging and requires specific tools available only in reference centers; therefore, some cases may remain undiagnosed or misdiagnosed. Modern hematology analyzers provide new erythrocytes (RBC) and reticulocytes (RET) parameters; however, few studies focused on these parameters and CHAs. Algorithms have been published on Sysmex XE and XN analyzers only for hereditary spherocytosis (HS) (Mullier, 2011; Persijn, 2012; Bobée, 2018; Sottiaux, 2020) and pyruvate kinase deficiency (PKD) (Bobée, 2018)

Aim. We aimed to create a comprehensive algorithm for screening and differential diagnosis of the most common forms of CHA (HS, PKD, and congenital dyserythropoietic anemia type II - CDAII) by using RBC and RET parameters provided by Sysmex XN. An additional algorithm for patients with methemoglobinemia due to NADH diaphorase deficiency or unstable hemoglobins (MetHb) was defined thanks to a particular scatterplot of the WBC differential channel observed in these patients.

Methods. Between 2020 and 2023, 500 consecutive patients were analyzed on Sysmex XN. Among them 305 had a confirmed diagnosis of CHA (106 HS, 21 PKD, 12 CDAII; 10 stomatocytosis, 118 hemoglobinopathies; 38 other CHAs), 6 had MetHb and 189 had acquired anemias (50 autoimmune hemolytic anemias; 32 myelodysplastic syndromes; 107 other anemias). When possible, patients were stratified based on the severity of anemia (Hb≤12 and Hb>12 g/dL). Parameters with significant differences (Mann Whitney U-test, p-value<0.05) and with the highest diagnostic power to identify patients with HS, PK, CDAII or MetHb (assessed by ROC curve) were combined into algorithms to discriminate each disease. Algorithms were validated on 1965 routine samples analyzed from Dec 2023 to Jan 2024, including 14 HS, 1 CDAII and 1 PKD newly diagnosed patients.

Results. All MetHb patients showed low values of NE-SFL, LY-Y, and MO-Y (mean fluorescence of NEUT, LYMPH and MONO respectively). All CDAII patients showed increased values in RDW-SD, hyperchromic (HyperHe) and macrocytic (MacroR) RBCs, and a decrease in Reticulocyte Production Index (RPI) and RET maturation indices (IRF, MFR). PKD patients showed high RET count (x10^9/L) and increased IRF combined with low values of hypochromic (HypoHe) and microcytic (MicroR) RBCs. HS patients had high RET count without equally elevated IRF along with increased MicroR and low HypoHe, leading to increased values of RET/IRF and MicroR/HypoHe ratios.

Below are the algorithms for MetHb, CDAII, PKD, HS listed by evaluation order and the diagnostic performances obtained (SE sensitivity; SP specificity; NPV negative predictive value; PPV positive predictive value). Square brackets group all the conditions that must be satisfied.

MetHb [NE-SFL<42.0; LY-Y<57.0; MO-Y<90; NRBC<5.0%; WBC>1.00x10^9/L] (SE 1.00, SP 1.00, NPV 1.00, PPV 1.00)

CDAII [RDW-SD>52.9; MacroR>3.8%; HyperHe>0.5%; MFR<9.5%; RPI<1.7; IRF<14.2%; Hgb<13.0; NRBC>0.0%; PLT>120x10^9/L] (SE=1.00, SP=0.99, NPV 1.00, PPV 0.92)
PKD with Hb≤12g/dL: [IRF>50%] OR [RET>95; IRF>25.0%; MicroR<1.7%;HypoHe<0.3%]; PKD with Hb>12g/dL: [RET>135; IRF>15.5%; MicroR<1.9%; HypoHe<0.2%] (SE 0.86, SP 0.99; NPV 0.99, PPV 0.78)

HS Precondition:[RET>75; RET/IRF≥7.7] HS with Hb≤12g/dL: [RET>150; MicroR/HypoHe 1.5 to 5.0; MicroR>2.0%; NRBC<5.0%;] OR [MicroR/HypoHe>5.0; MicroR>1.5%] HS with Hb>12: [RET/IRF>15.0] OR [RET>190; RET/IRF 9.0 to 15.0; MicroR≥2.5%; MicroR/HypoHe>5.0] (SE 1.00, SP 0.96 NPV 1.00, PPV 0.87)
The excellent diagnostic performances have been confirmed on the 1965 samples used for validation; all new patients with HS, CDAII and PKD were correctly classified. The algorithms published by the other authors, tested on the 500 patients of this study, showed lower SE for both HS (0.85 to 0.92) and PKD (0.14)
Conclusions. For the first time we present a comprehensive algorithm for HS, CDAII, PKD and MetHb with excellent performance and higher diagnostic accuracy than previously published algorithms for HS and PKD. It can represent a simple, costless, and efficient first-line screening of the most common CHAs even outside reference centers, contributing to early diagnosis and better management of these patients.

Disclosures Bianchi: *Agios Pharmaceuticals:* Membership on an entity's Board of Directors or advisory committees. **Passamonti:** *Kyowa Kirin:* Honoraria, Speakers Bureau; *Karyiopharma:* Honoraria, Speakers Bureau; *GSK:* Honoraria, Speakers Bureau; *AOP:* Honoraria, Speakers Bureau; *Janssen:* Honoraria, Speakers Bureau; *Abbvie:* Honoraria, Speakers Bureau; *BMS/Celgene:* Honoraria, Speakers Bureau; *Novartis:* Honoraria, Speakers Bureau; *MEI:* Honoraria, Speakers Bureau; *Sumitomo:* Honoraria, Speakers Bureau; *Kartos Therapeutics Inc.:* Honoraria, Speakers Bureau. **Barcellini:** *Sobi:* Consultancy; *Sanofi:* Consultancy, Honoraria, Speakers Bureau; *Alexion, AstraZeneca Rare Disease:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Novartis:* Consultancy, Honoraria, Speakers Bureau.

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