

BIO MED 2024

LES JOURNÉES POUR L'AVENIR DE LA BIOLOGIE MÉDICALE

JEUDI 23 &
VENDREDI 24
MAI 2024

Les indices érythrocytaires: actualités

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Paris, le 23 mai 2024

Déclaration des conflits d'intérêts

Aucun conflit d'intérêt

Mosaïque d'Ulysse et les sirènes (Musée National du Bardo, Tunis-TUNISIE)



Découverte à DOGGA (Nord-Ouest de la TUNISIE) , datée du III^{ème} siècle

Paramètres classiques et MOINS classiques...

(RBC/PLT channel)

Parameter (unit)	What?	Channel
MCV (fL)	Mean corpuscular volume = hematocrit/#RBC	RBC/PLT channel
MCH (pg)	Mean cell haemoglobin content = hemoglobin/#RBC	RBC/PLT channel
MCHC (g/dL)	Mean cell haemoglobin concentration = haemoglobin/hematocrit	RBC/PLT channel
RDW-CV (%) or RDW-SD	Red cell distribution width	RBC/PLT channel

(RBC/PLT channel)

MicroRBC* (%)	Percentage of microcytic red cells (volume < 60 fL)	RBC/PLT channel
MacroRBC* (%)	Percentage of macrocytic red cells (volume > 120 fL)	RBC/PLT channel

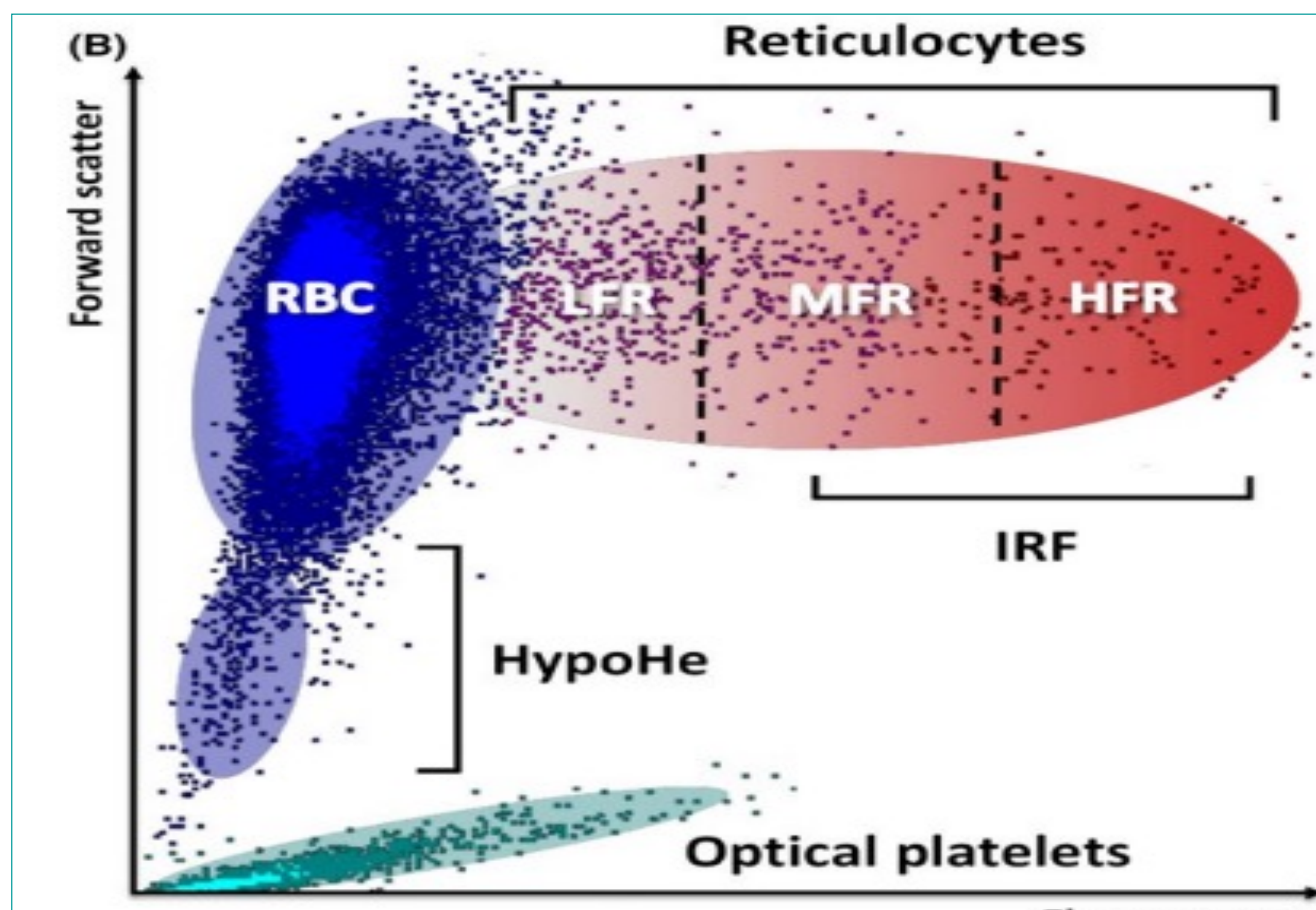
R-MFV L-MCV S-MCV

(RET channel)

Parameter (unit)	What?	Channel
Reticulocytes (#/L or %)	Number of reticulocytes	RET channel
Ret-He (pg)	Reticulocyte hemoglobin equivalent	RET channel
Delta-He* (pg)	Difference in hemoglobin content between RBC and reticulocytes	RET channel
Hypo-He* (%)	Percentage of hypochromic RBC (cellular haemoglobin content lower than 17 pg)	RET channel
Hyper-He* (%)	Percentage of hyperchromic red cells (cellular haemoglobin content higher than 49 pg)	RET channel
IRF (%)	Immature reticulocyte fraction = medium fluorescence reticulocytes (MFR) + high fluorescence reticulocytes (HFR)	RET channel
FRC* (#/μL or %)	Percentage of fragmented RBC	RET channel
RBC score*	$1/(1 + \exp(-(-7.6055 + 1.5873 * \text{FRC}(\%) + 0.0402 * \text{Reticulocytes } 10^9 / \text{L})))$	RET channel

Canal RET

Réticulocytes: *what else?*



- LFR (réticulocytes à faible fluorescence) - réticulocytes « matures »
- MFR (réticulocytes à fluorescence moyenne) - réticulocytes « semi-matures »
- HFR (réticulocytes à haute fluorescence) - réticulocytes « immatures »

% rétic

IRF

RPI

Correction des rétic si « anémie sévère »

$$RPI = \frac{RET [\%]}{\text{Temps de maturation RET dans le sang en jours}} \times \frac{HCT [L/L]}{0,45 \text{ (Standard HCT)}}$$

BIO MED 2024

LES JOURNÉES POUR L'AVENIR DE LA BIOLOGIE MÉDICALE

JEUDI 23 &
VENDREDI 24
MAI 2024

6 cas cliniques

janvier 2024- mai 2024

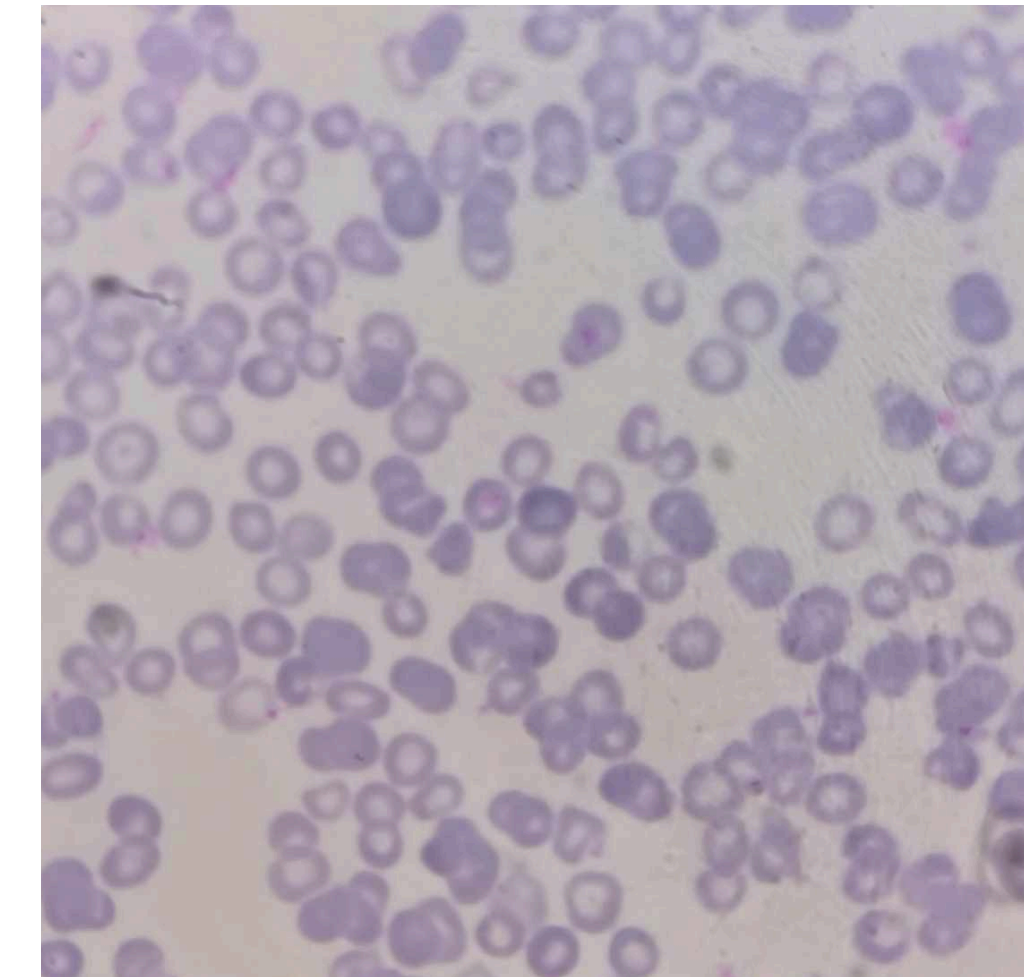
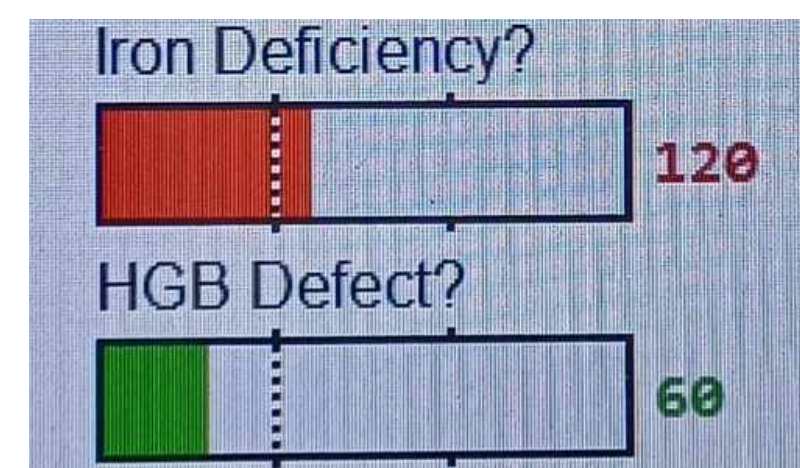
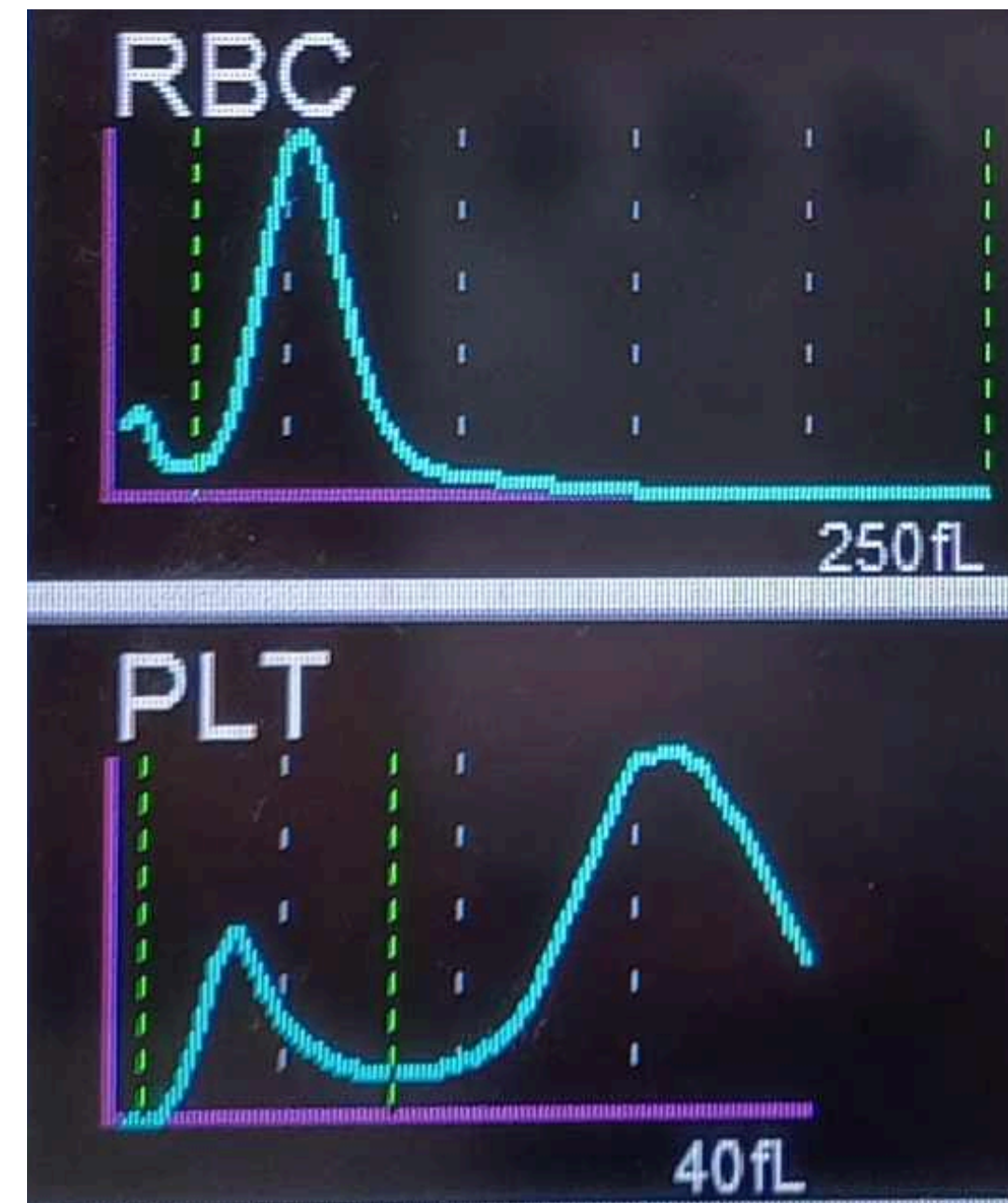


Cas 1

Nourrisson âgé de 10 mois, fatigue pendant la tétée et le biberon

Anémie hypochrome microcytaire avec anisocytose

RBC	5.34	$10^6/\mu\text{L}$
HGB	8.7	g/dL
MCV (VGM)	60.9	fL
MCH (TCMH)	16.3	pg
MCHC (CCMH)	26.8	g/dL
RDW-CV	22.9	%
Micro-R	52.9	%
Ret %	1.02	%
Ret	54 500	$/\text{mm}^3$
RET-He	13.7	pg
Hypo-He	73.5	%

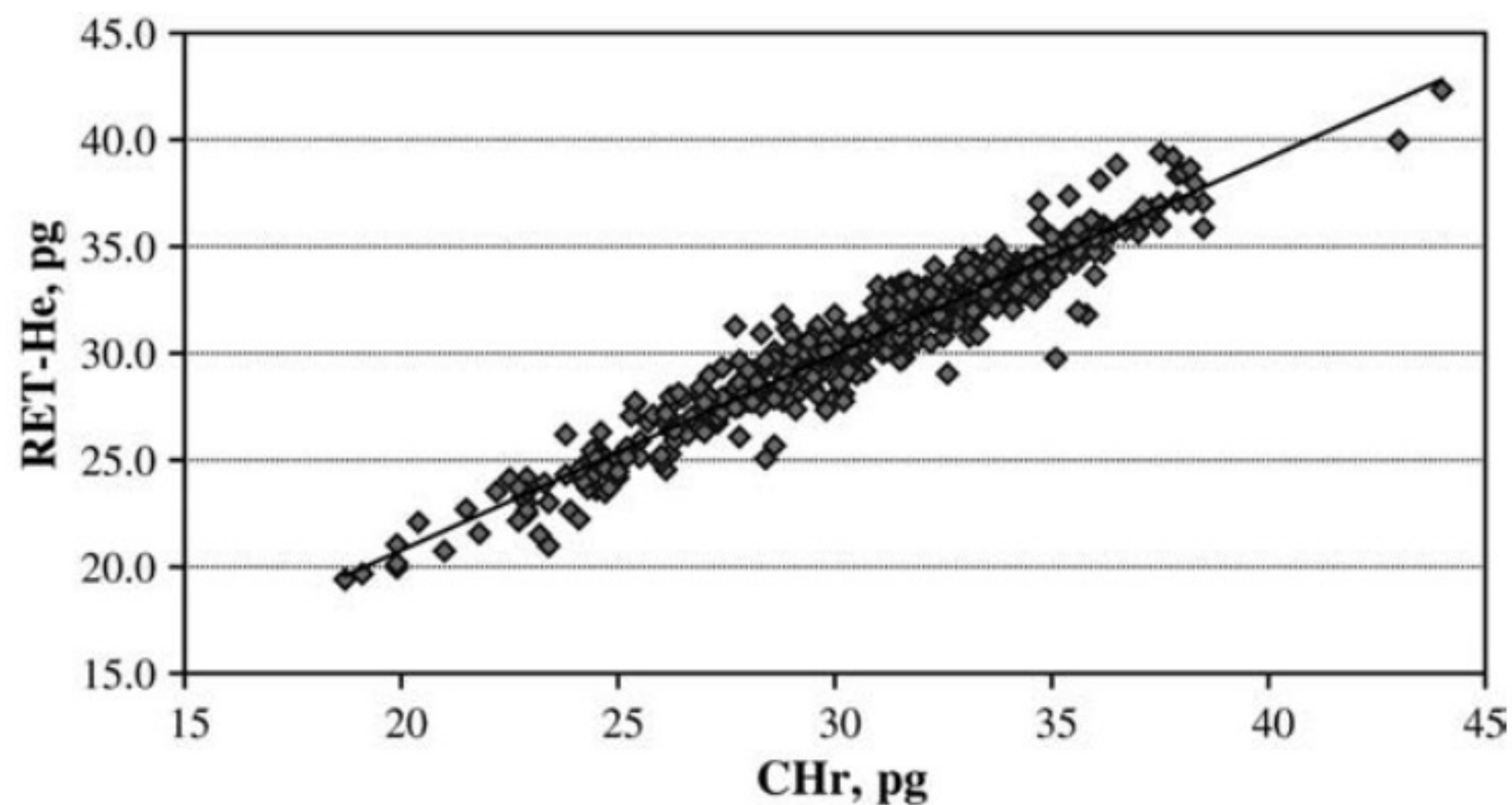


- Anémie ferriprive (AF)
- β -thal HMZ
- AF + β -thal HTZ
- ...

Reticulocyte hemoglobin measurement – comparison of two methods in the diagnosis of iron-restricted erythropoiesis

RET-He et CHr décrivent le « *même phénomène* »

Systemex XE/XN Siemens Advia

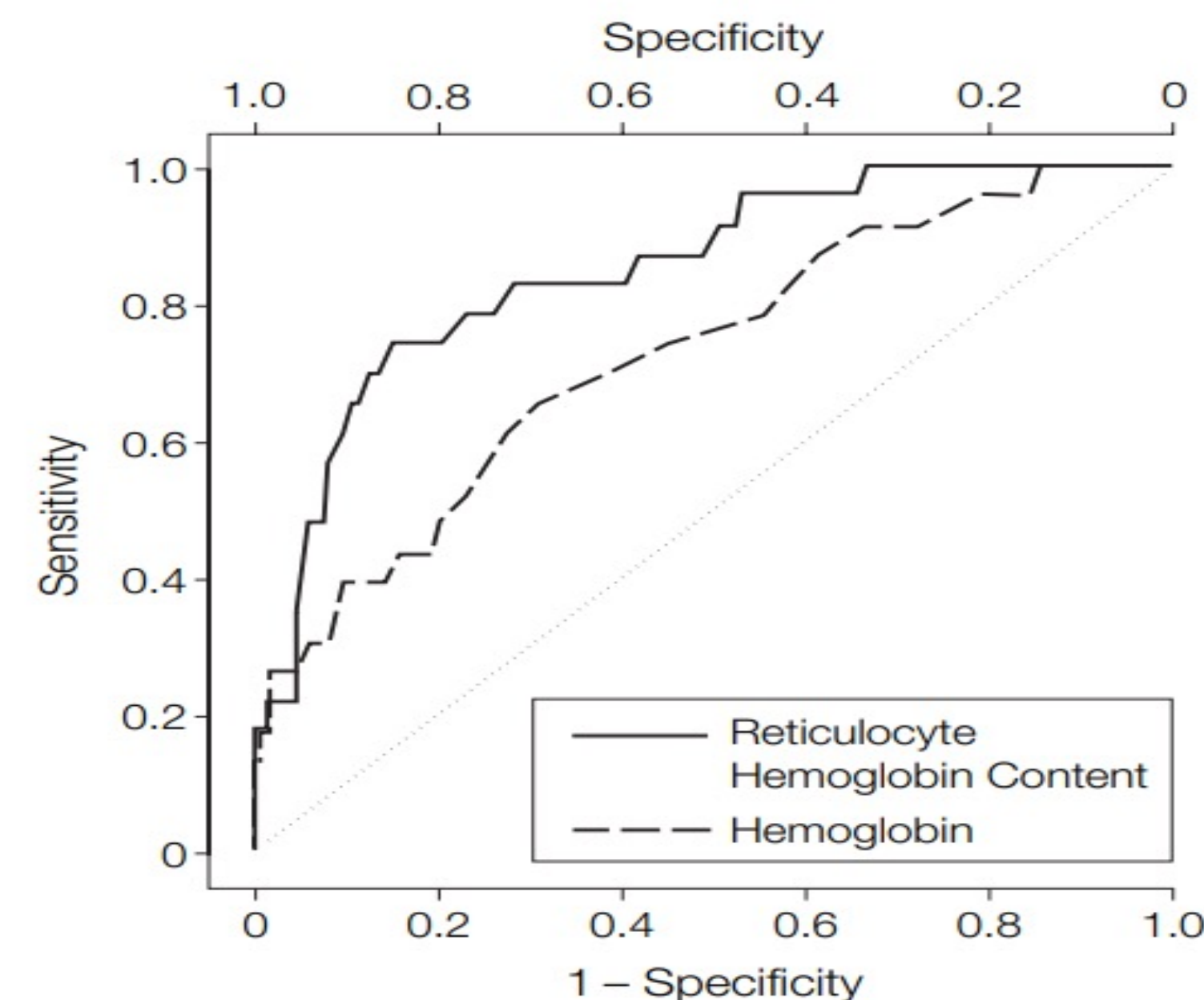


Ret-He/CHr* et détection de la carence martiale chez le nourrisson

- ✓ 202 nourrissons entre 9 – 12 mois, en bonne santé avec une Hb > 11 g/dL (Boston)
- ✓ **Ret-He/CHr < 27.5 pg** : dépistage des nourrissons avec carence martiale sans anémie
- ✓ **Ret-He/CHr < 27.5 pg**: associé à une anémie lors du 2^{ème} dépistage au cours de la 2^{ème} année de vie des nourrissons.

Seuil RET-He/CHr* 27.5 pg
(sp 72%; Sb 83%, VPN 97%)

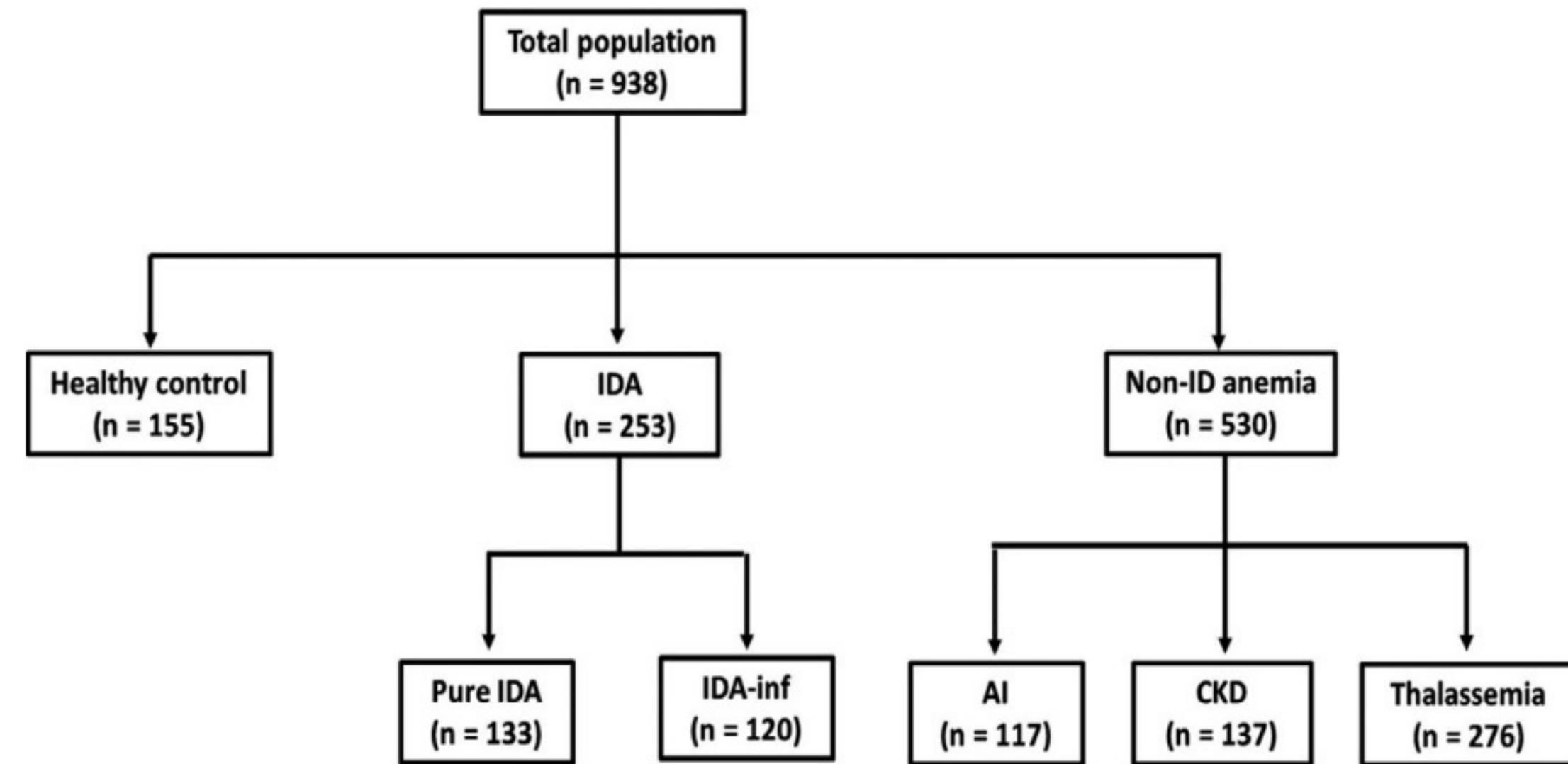
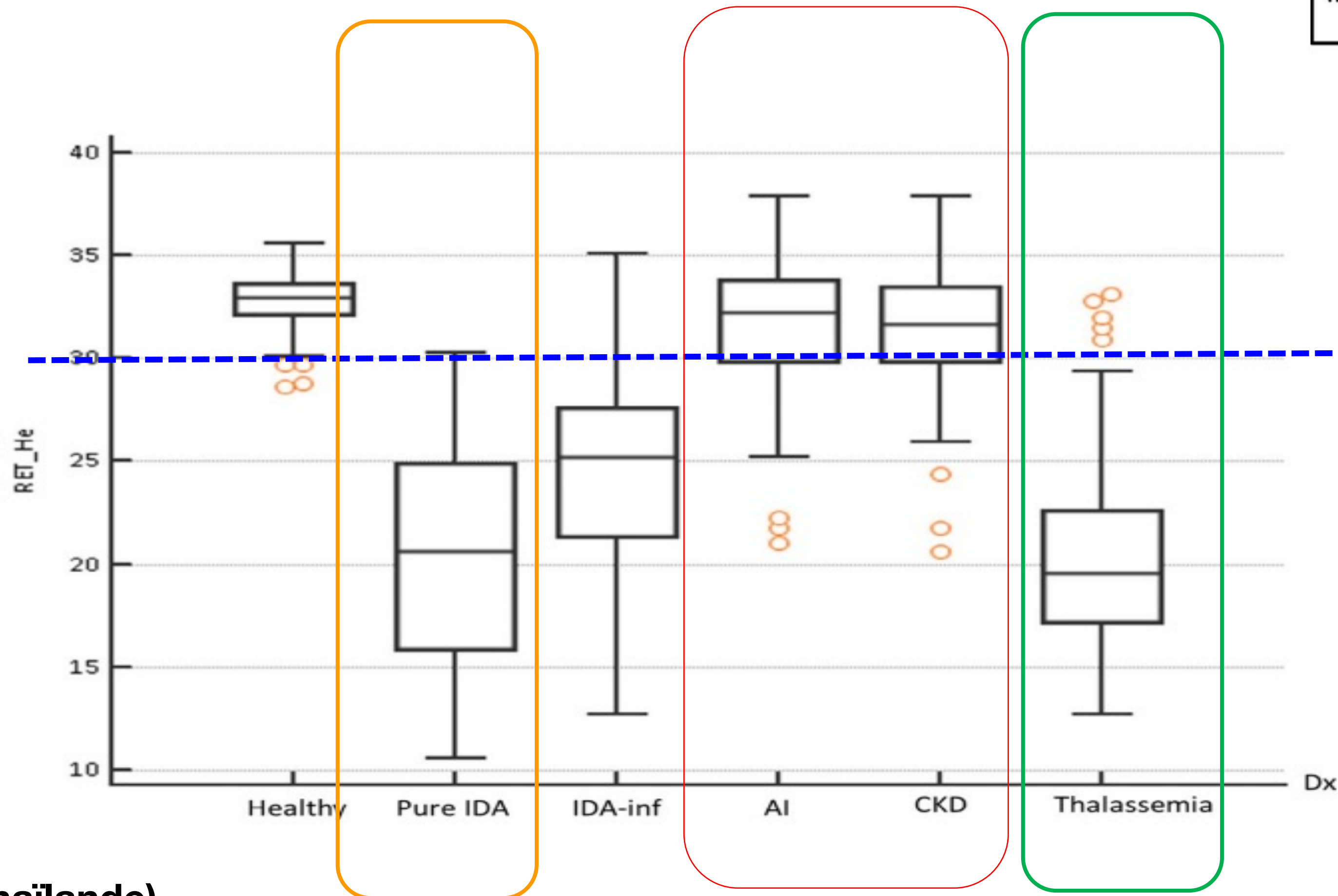
Figure 3. Receiver Operating Characteristic Curves for Reticulocyte Hemoglobin Content and Hemoglobin for the Detection of Iron Deficiency at Initial Screening



RET-He > 30 pg:

exclusion d'une AF

(Sp 96%; Sb 97.4%, VPP 80%; VPN 99.6%)



RET-He ≤ 30 pg:

-AF

-AF+ AI

-Thalassémie

RET-He > 30 pg:

-AI

-maladie rénale chronique

RET-He/ CHr

Avantage

- *rapide*
- *pratique*
- *rentable*



Intérêts/indications

- *disponibilité du fer pour l'érythropoïèse dans la moelle*
- *Indicateur précoce de la carence (**demi-vie courte des réticulocytes**)*
- *indicateur précoce de la réponse au traitement martial (**dès le 2^{ème} jour**)*

- *NE différencie PAS entre une AF et une thalassémie*
- *N'est PAS influencé par l'inflammation*

Anciennes formules de discrimination entre AF/ β -thal

Table 1. Overview of discrimination formulas including cut-off levels for iron deficiency anemia and for β -thalassemia.

Authors	Formulas	Iron deficiency anemia	Thalassemia
England & Fraser ¹⁴	$MCV - RBC - 5 \times Hb - 3.4$	>0	<0
Green & King ¹⁵	$(MCV^2 \times RDW - CV) / (Hb \times 100)$	>65	<65
Mentzer ¹³	MCV/RBC	>13	<13
Shine & Lal ¹⁶	$MCV^2 \times MCH \times 0.01$	>1530	<1530
Ricerca ¹⁷	RDW/RBC	>4.4	<4.4
Shrivastava ¹⁸	MCH/RBC	>3.8	<3.8

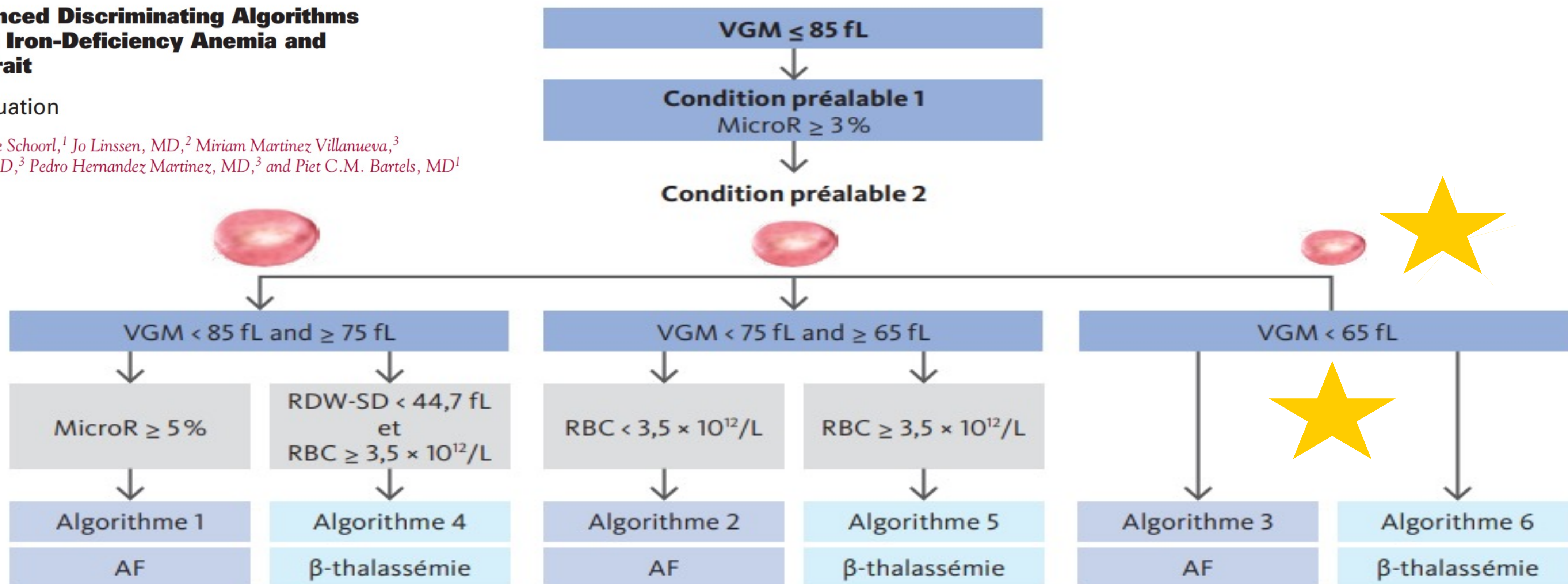
MCV, mean red blood cell volume; RBC, red blood cell; Hb, hemoglobin; RDW-CV, red blood cell distribution width; MCH, mean red blood cell hemoglobin.

***Mentzer 11.4**

Efficacy of Advanced Discriminating Algorithms for Screening on Iron-Deficiency Anemia and β -Thalassemia Trait

A Multicenter Evaluation

Margreet Schoorl,¹ Marianne Schoorl,¹ Jo Linssen, MD,² Miriam Martinez Villanueva,³ José A Velasco NoGuera, MD,³ Pedro Hernandez Martinez, MD,³ and Piet C.M. Bartels, MD¹



n°	Algorithme	Condition
1	MicroR / HYPO-He < 4,0 et $[(VGM^2 \times RDW-CV) / (HGB \times 100)] \geq 75$ et RET < 0,08	AF
2	MicroR / HYPO-He < 3,4 et $[(VGM^2 \times RDW-CV) / (HGB \times 100)] \geq 77$ et RET < 0,08	AF
3	MicroR – HYPO-He – RDW-CV < -5,2	AF
4	$[(VGM^2 \times RDW-CV) / (HGB \times 100)] < 75$ et $[VGM - RBC - 3,4 - (5 \times HGB)] < 4,0$ et RET < 0,08 et Delta-He ≥ 0	β -thal
5	$[(VGM^2 \times RDW-CV) / (HGB \times 100)] < 77$ et MicroR / HYPO-He $\geq 2,0$	β -thal
6	MicroR – HYPO-He – RDW-CV $\geq -5,2$	β -thal

Cas 1

Nourrisson âgé de 10 mois, fatigue pendant la tétée et le biberon

RBC	5.34	10 ⁶ /μL
HGB	8.7	g/dL
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RDW-CV	22.9	%
Micro-R	52.9	%
Ret %	1.02	%
Ret	54 500	/mm ³
RET-He	13.7	pg
Hypo-He	73.5	%

HypoHe	73.5	%
RDW-CV	22.9	%
Micro-R	52.9	%

MicroR – HypoHe – RDWCV

$$= = -43.5 < -5.2$$

AF

Algorithmme 3

Nouvelles formules/algorithmes de discrimination entre AF/ β -thal

Reference	Iron Deficiency					Thalassemia				
	AUC	Sensitivity (%)	Specificity (%)	Disease Prevalence 10%		AUC	Sensitivity (%)	Specificity (%)	Disease Prevalence 4%	
				PPV (%)	NPV (%)				PPV (%)	NPV (%)
England and Fraser ¹	0.696	97	45	10	90	0.918	51	96	36	98
Green and King ²	0.691	97	56	15	90	0.945	64	97	44	99
Mentzer ³	0.613	94	47	4	90	0.854	48	95	30	98
Urrechaga ²⁰	0.791	76	83	53	93	0.806	84	68	10	99
Urrechaga et al ²¹	0.828	90	68	54	93	0.893	69	89	21	99
Algorithm 1-3 for discrimination of IDA	0.878	79	97	74	98					
Algorithm 4-6 for discrimination of β -thalassemia						0.860	74	98	75	99

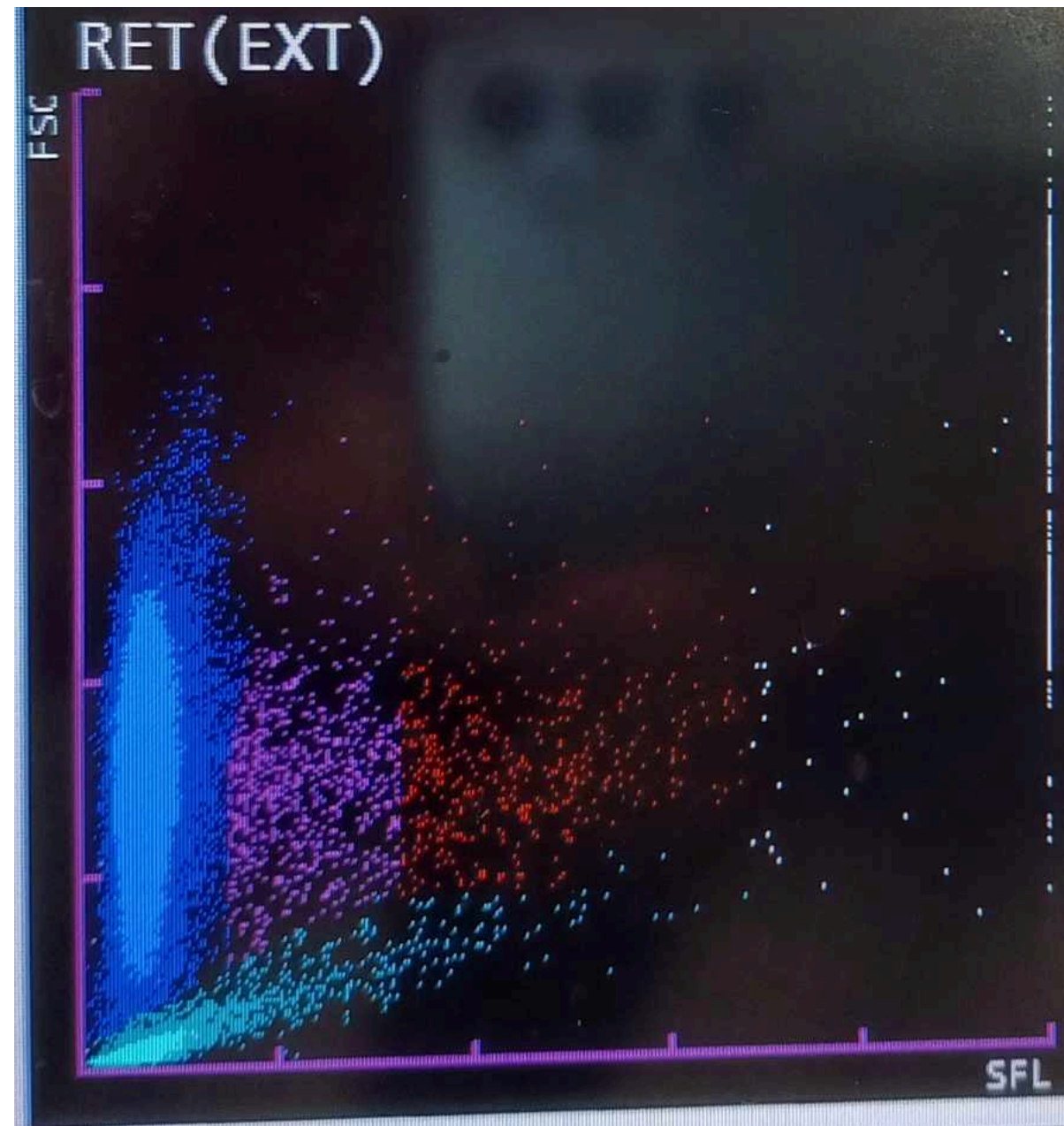
142 AF, 34 β -thalassémies et 309 sujets sains

Schoorl M et al. 2012. Am. J. Clin. Pathol. 138(2): 300–304.

Amélioration des performances diagnostiques

Cas 1

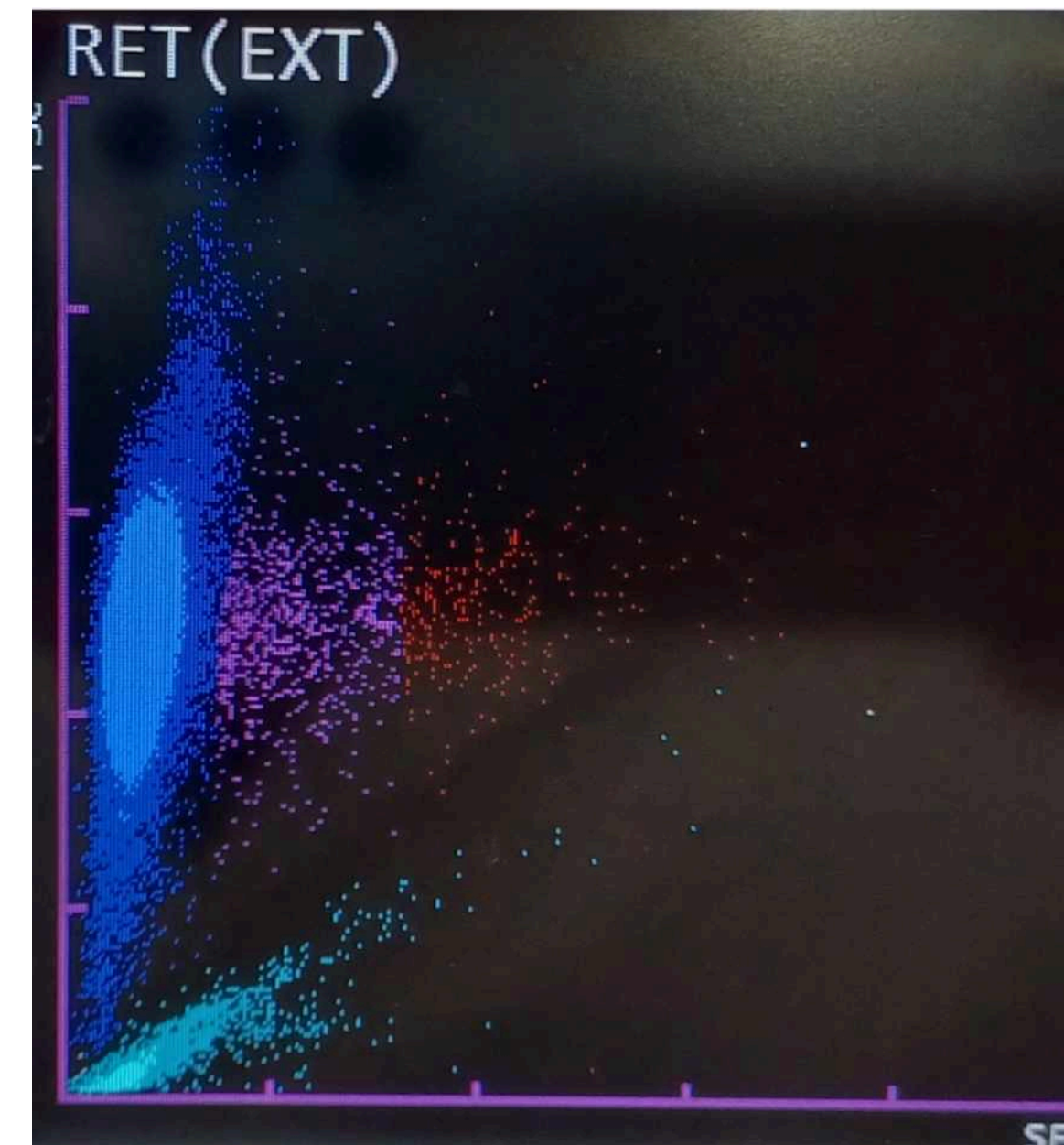
AF



Hypo-He	95.4	
IRF	30.9	%
LFR	69.1	%
MFR	20.5	%
HFR	10.4	%

Autre patiente du labo

β Thal HTZ



Hypo-He	29.7	
IRF	17.4	%
LFR	82.6	%
MFR	12.3	%
HFR	5.1	%

Cas 2

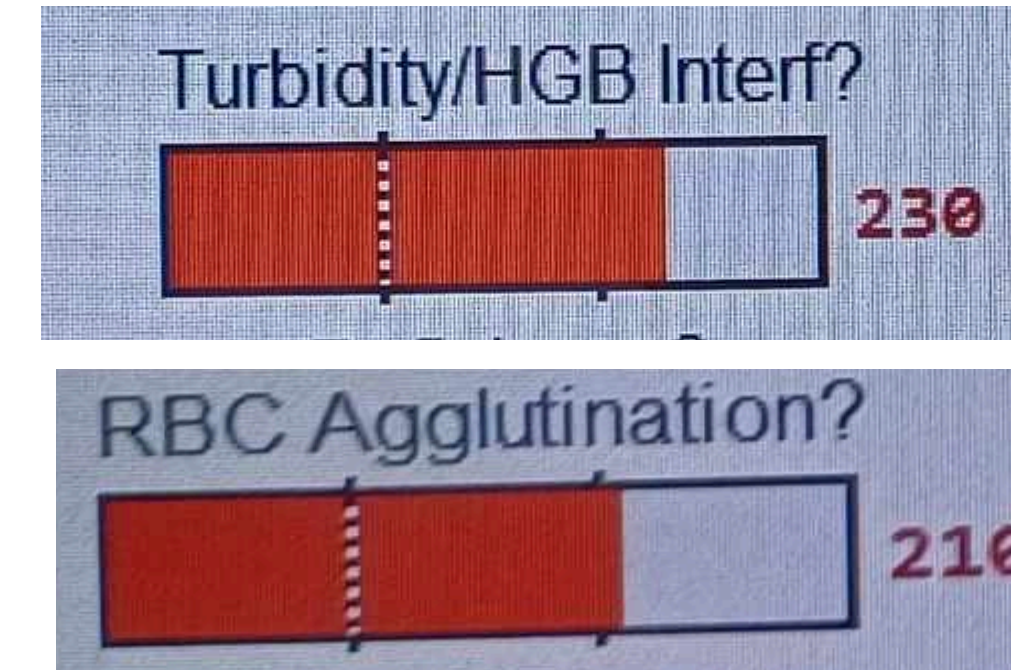
Femme, KM, 61 ans, consultation de gynécologie, pré-AG

TECHNIQUE

impédance
(classique)

Impédance après
incubation 1h à 37°C

	1 ^{er} passage	2 ^{ème} passage
RBC (10 ⁶ /μL)	1.55	4.62
Hb (g/dL)	13.3	13.2
MCV (VGM) fL	107.1	93.3
CMH (TCMH) pg	90.3	30.1
CCMH (g/dL)	84.3	32.3
Hte (%)	16.6	42.1
RDW-CV (%)	14.2



S-MCV 90.5 fL L-MCV 190.1 fL



Cas 2

Femme, KM, 61 ans, consultation de gynécologie, pré-AG

TECHNIQUES

impédance (classique)

Impédance après incubation 1h à 37°C

Optique (canal RET)

1^{er} passage

2^{ème} passage

P- mesurés

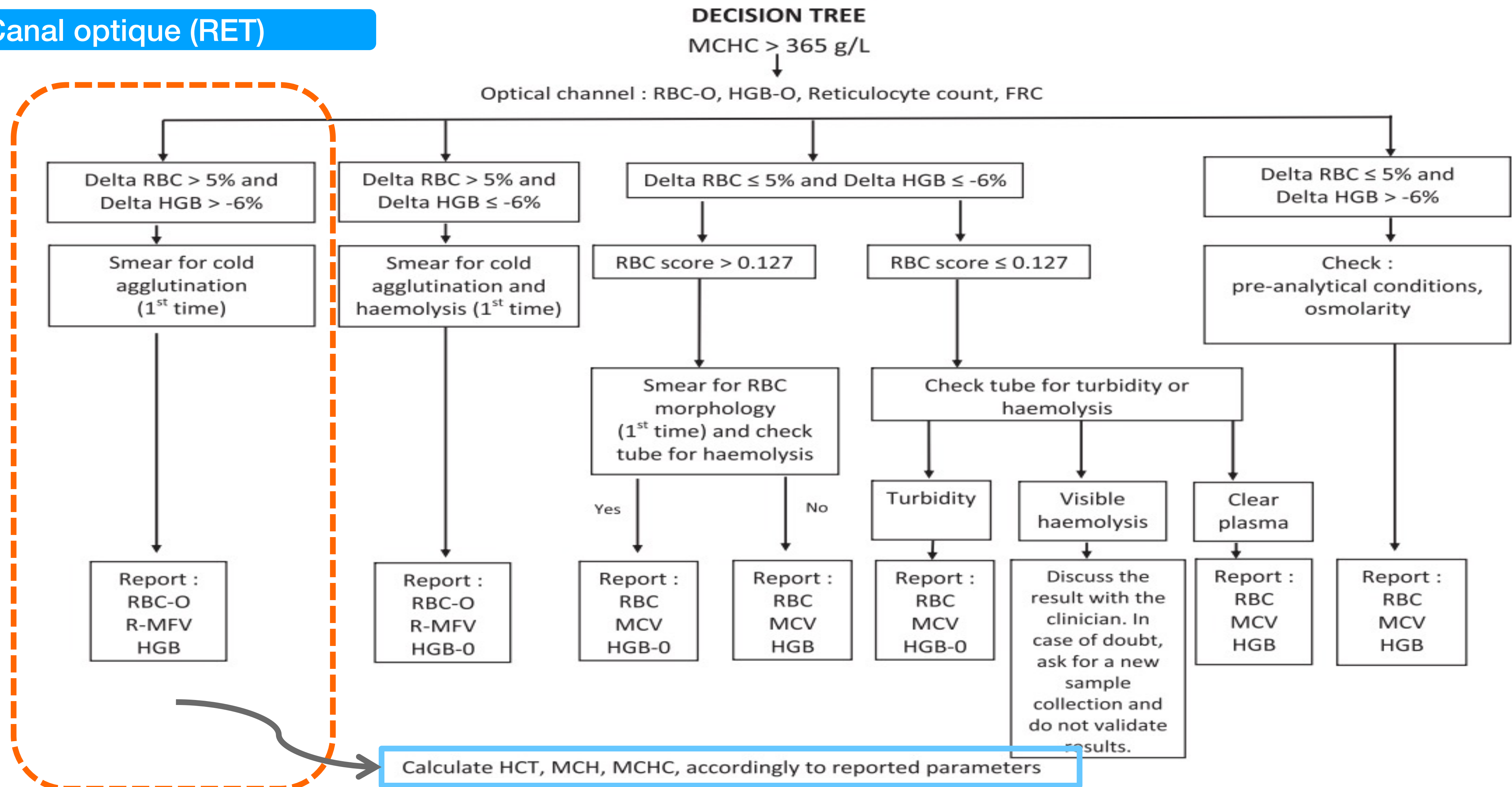
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Hte (%)	16.6
RDW-CV (%)

RBC (10 ⁶ /μL)	4.63
Hb (g/dL)	13.2
MCV (VGM) fL	93.3
CMH (TCMH) pg	30.1
CCMH (g/dL)	32.3
Hte (%)	42.1
RDW-CV (%)	14.2

RBC-O (10 ⁶ /μL)	4.62
RBC (10 ⁶ /μL)	1.55
Delta-RBC (%)	198
Hb-O (g/dL)	12.6
Hb (g/dL)	13.3
Delta-Hb (%)	-5.26
CCMH	75.3
MFV (fL)	90.5

CCMH > 36.5 g/dL

Canal optique (RET)



Cas 2

Femme, KM, 61 ans, consultation de gynécologie, pré-AG

TECHNIQUES

impédance (classique)

Impédance après incubation 1h à 37°C

Optique (canal RET)

1^{er} passage

2^{ème} passage

P- mesurés

P-calculés

RBC (10 ⁶ /μL)	1.55
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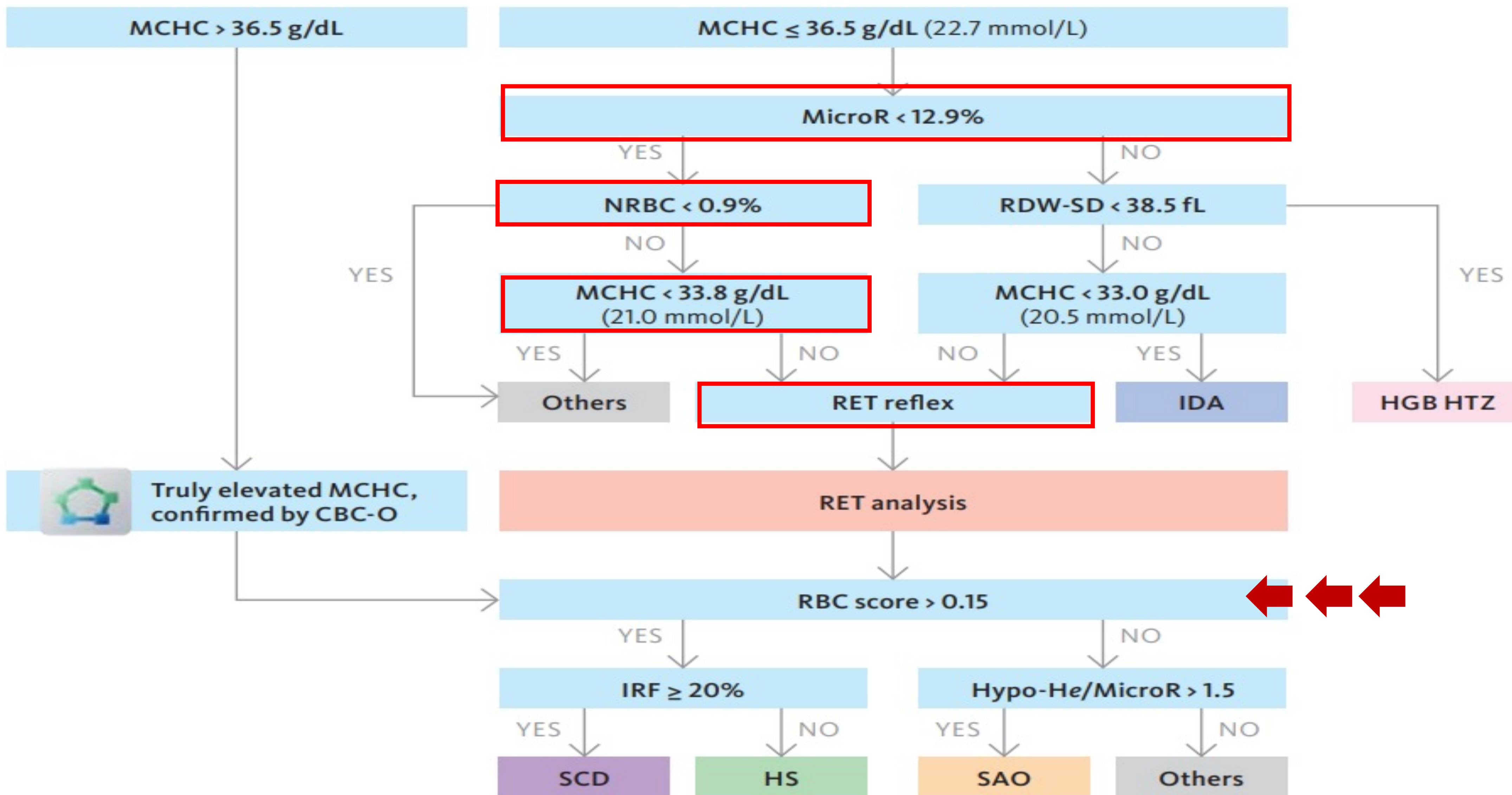
Htec (%)	41.8
TCMH _c (pg)	28.73
CCMH _c (g/dL)	31.81

Femme, IB, 24 ans, admise en HDJ Hto pour transfusion (dernière transfusion date de plus de 3 mois)

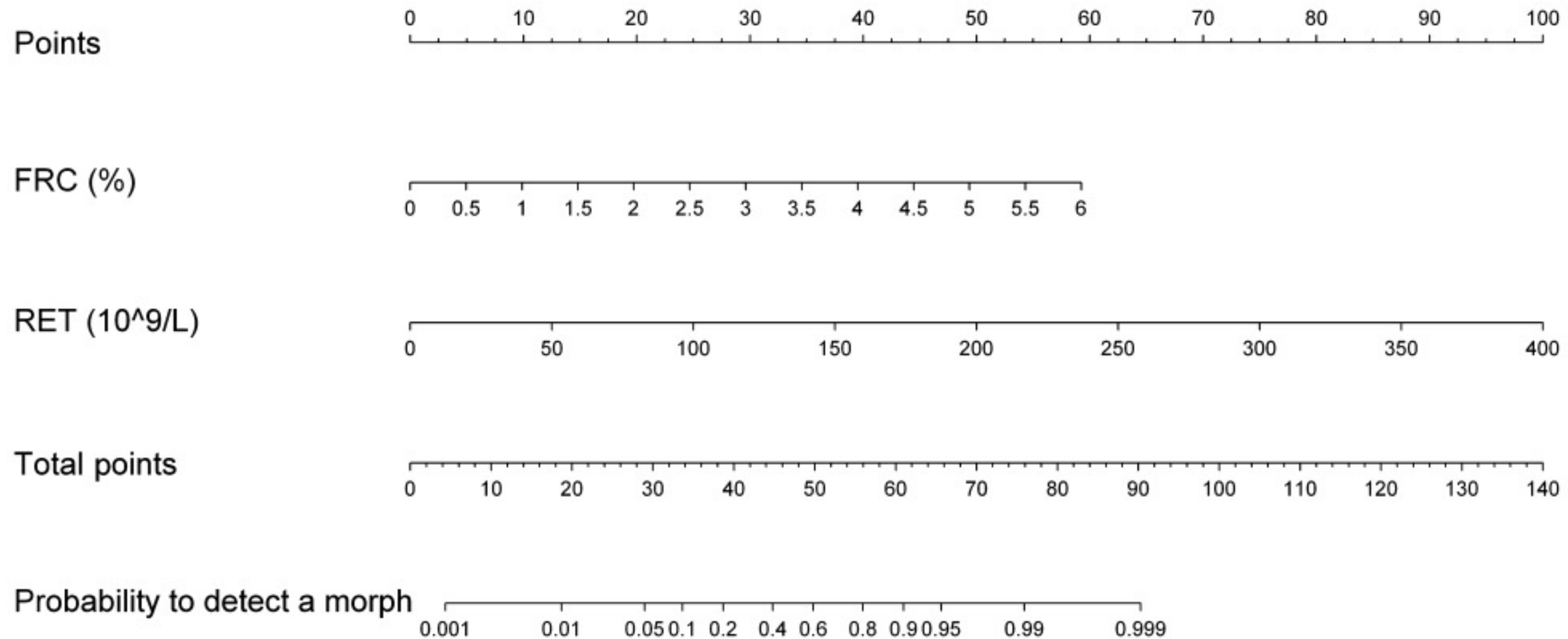
RBC	1.76	10 ⁶ /μL
HGB	6.2	g/dL
MCV (VGM)	96.6	fL
MCH (TCMH)	35.2	pg
MCHC (CCMH)	36.5	g/dL
RDW-CV	22.5	%
RET-He	31.3	pg
Micro-R	4.1	%
Hypo-He	2	%

NRBC	3.7	%
IRF	20.3	%
RET	324 000	/mm ³
RPI	5.1	%
TCD	-	
Bilan hémolyse	+	

Anémie hémolytique *non* immunologique



Nomogramme qui s'est traduit par le « RBC-score »

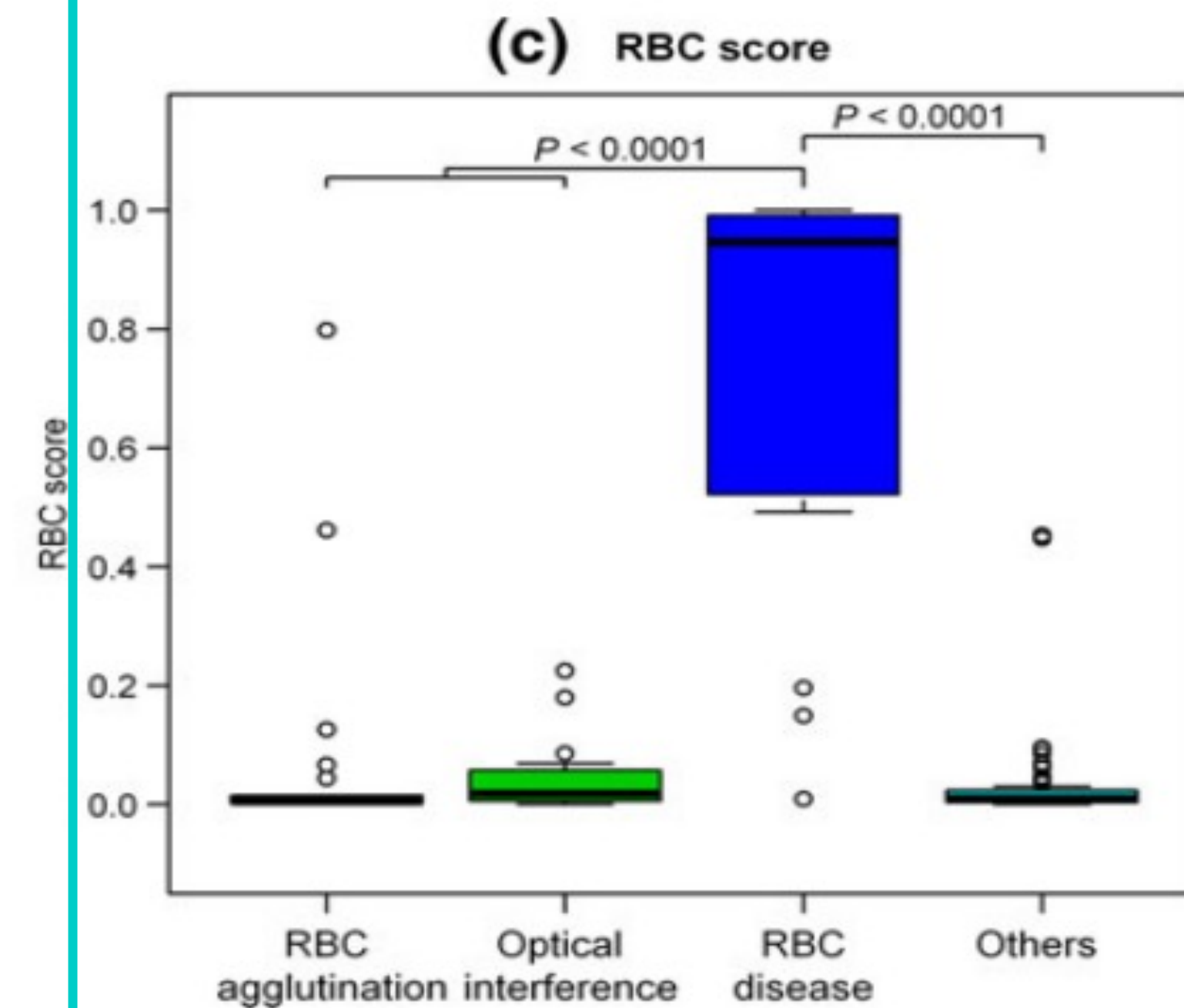
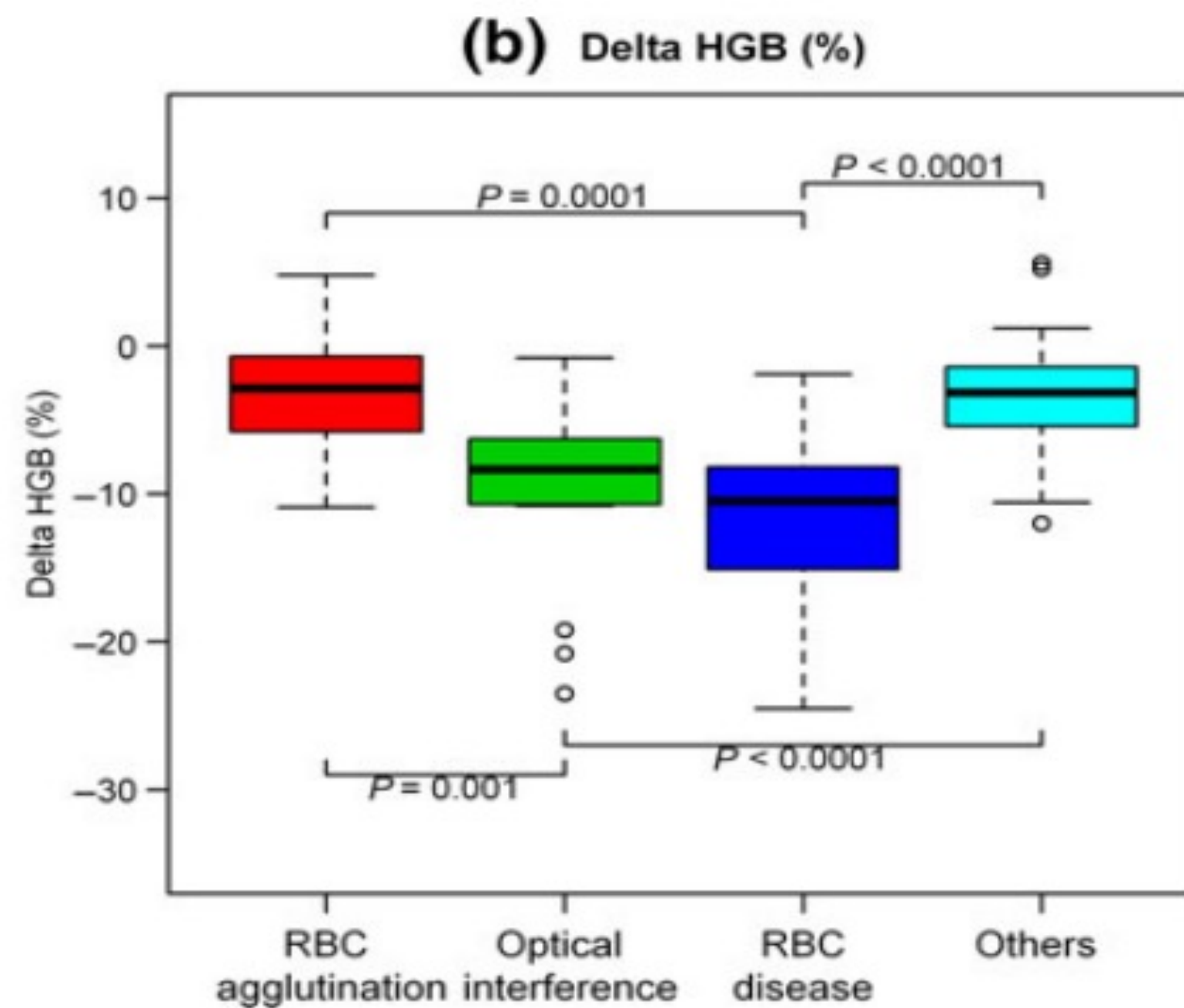
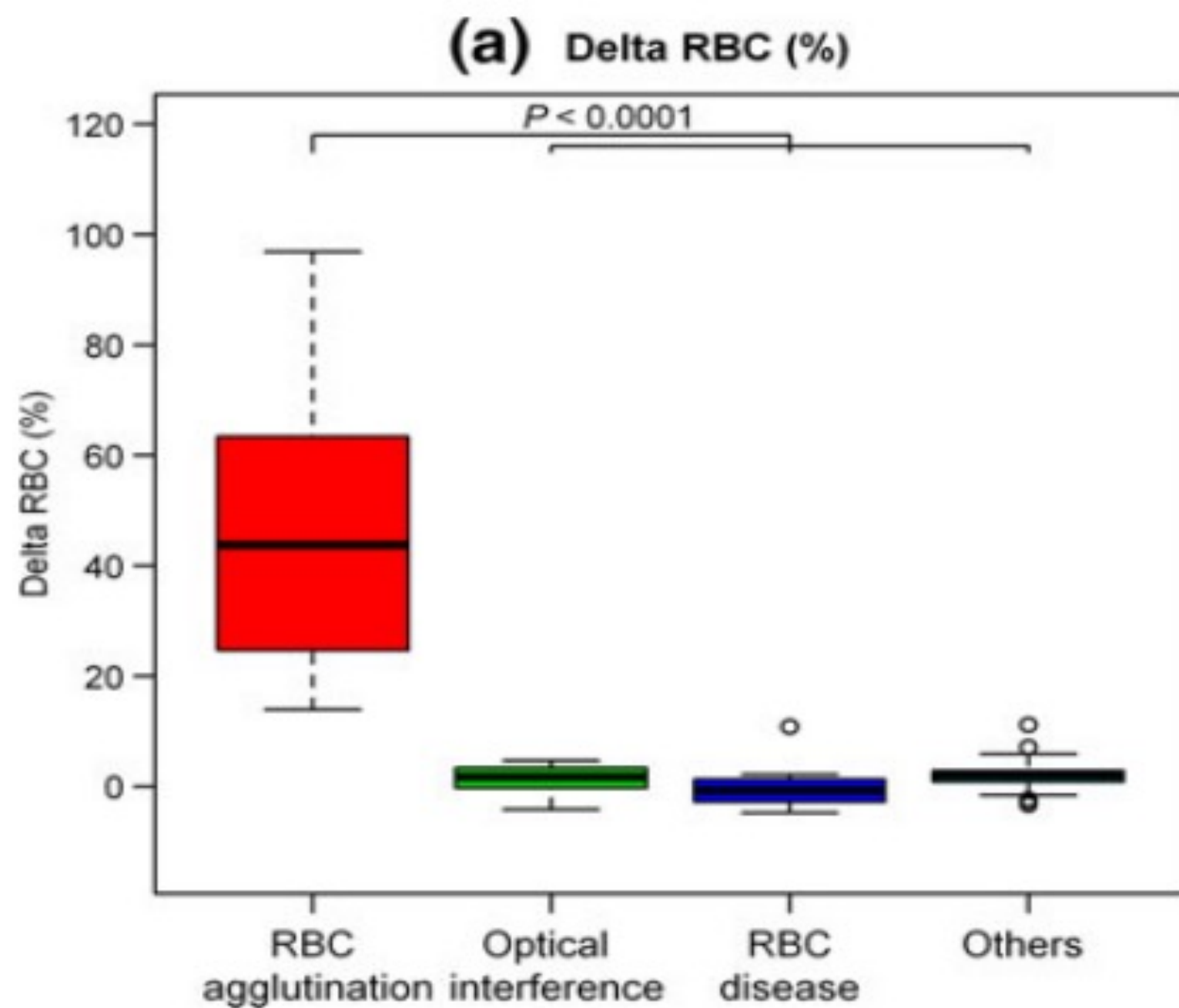


128 patients avec CCMH > 36.5 g/dL



Increased mean corpuscular haemoglobin concentration: artefact or pathological condition?

Y. BERDA-HADDAD*, C. FAURE*, M. BOUBAYA†, M. ARPIN*, S. COINTE*‡, D. FRANKEL*, R. LACROIX*‡, F. DIGNAT-GEORGE*‡



$$\text{delta RBC \%} = ((\text{RBCO} - \text{RBC}) / \text{RBC}) \times 100$$

$$\text{delta HGB \%} = ((\text{HGBO} - \text{HGB}) / \text{HGB}) \times 100.$$

Cas 3

Femme, IB, 24 ans, admise en HDJ Hto pour transfusion (dernière transfusion date de plus de 3 mois)

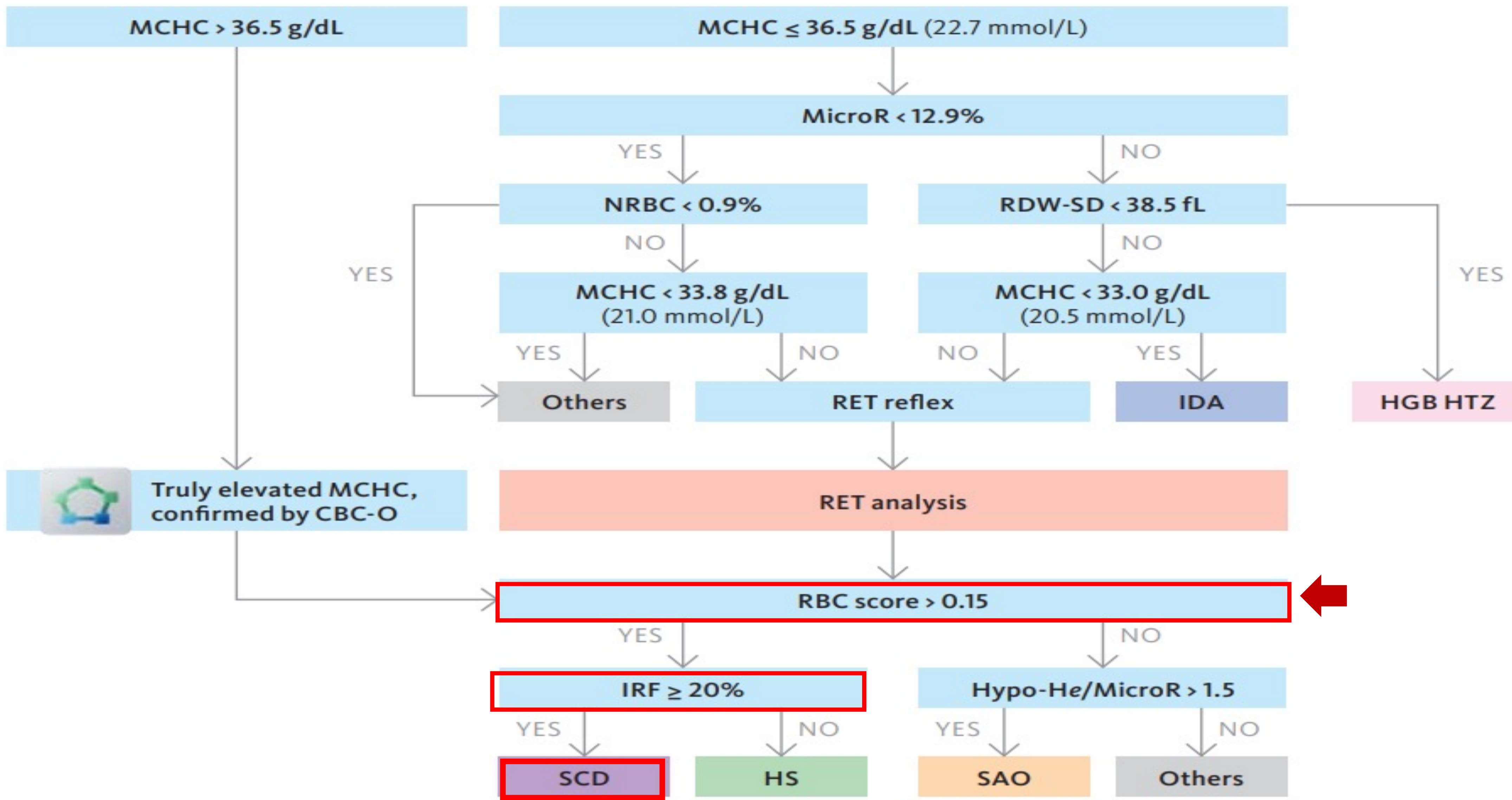
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Hypo-He	2	%

NRBC	3.7	%
IRF	20.3	%
RET	324 000	/mm ³
RPI	5.1	%

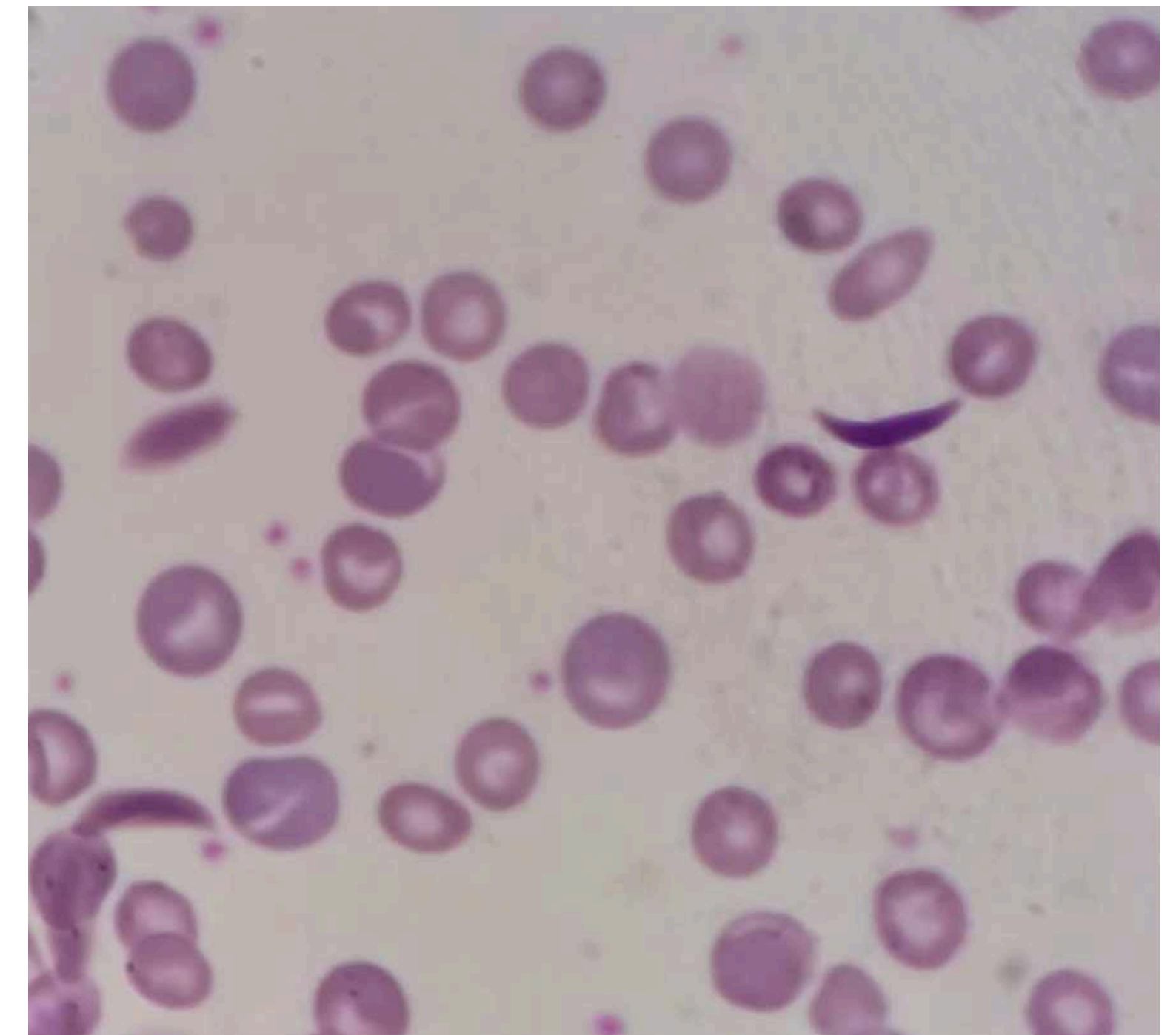
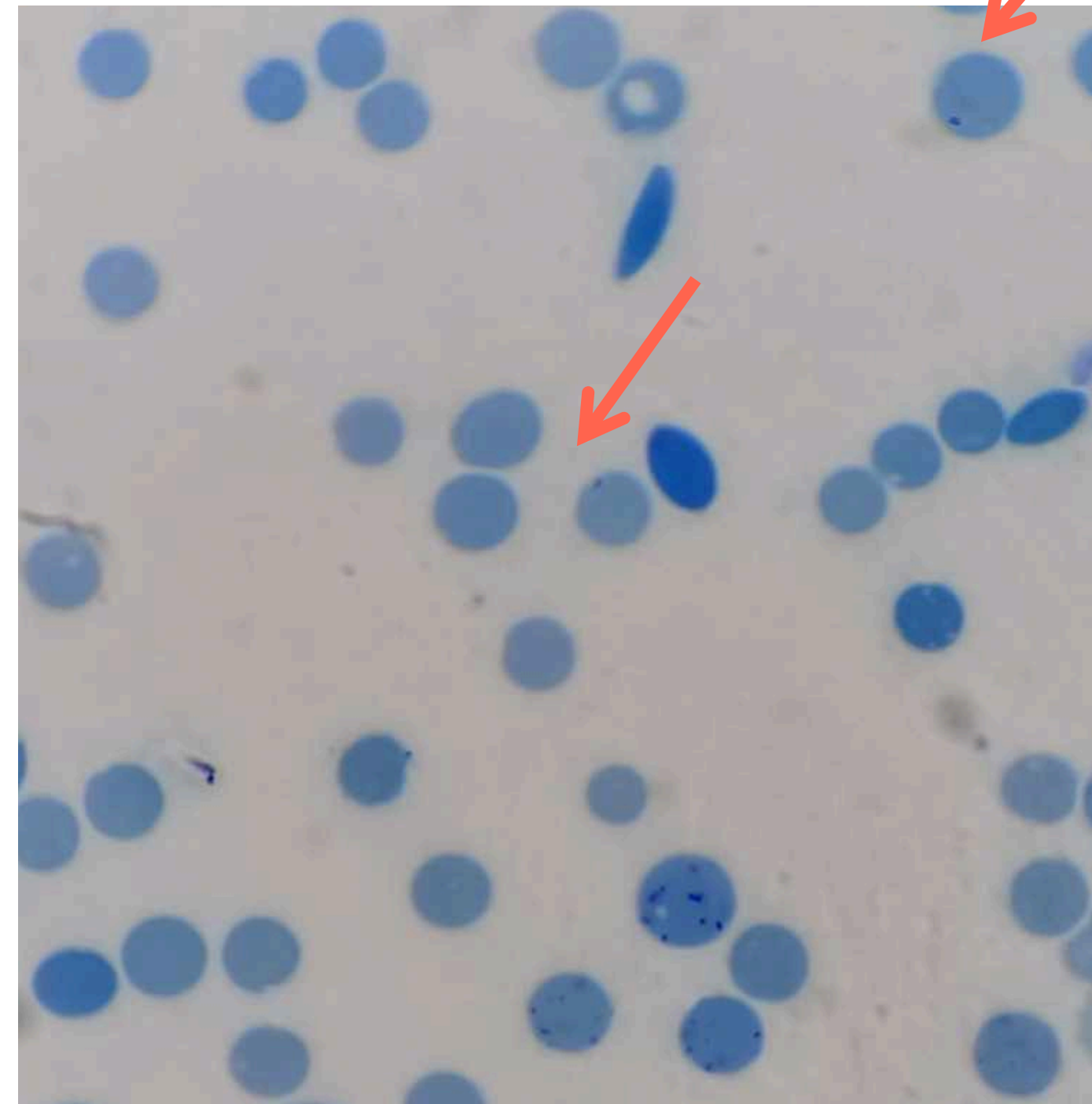
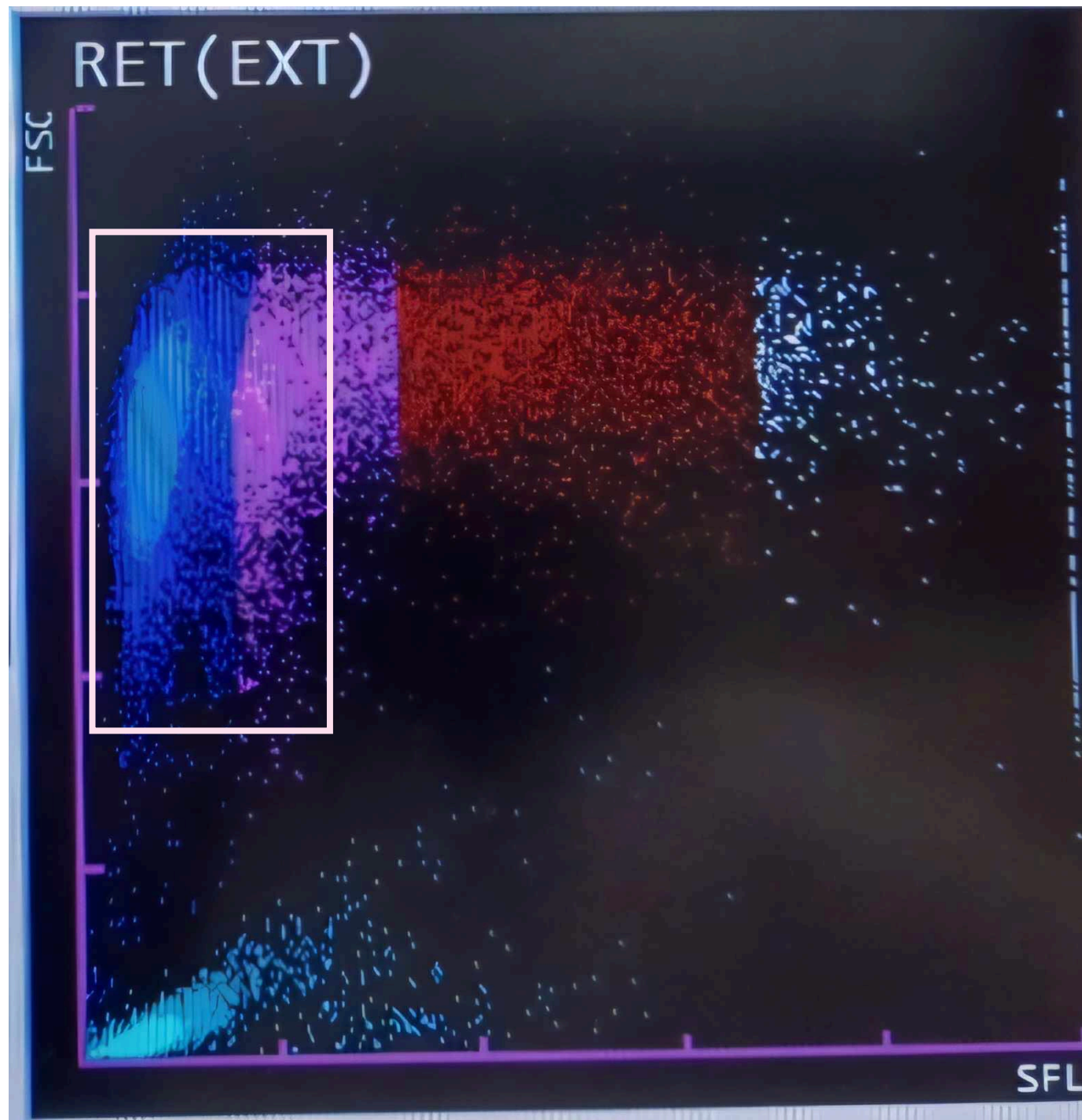
RBC Score =

$$\left[\frac{1}{1 + e^{-(-7.6055 + 1.5873 * FRC(\%) + 0.0402 * RET109 /L)}} \right]$$

RBC score = 0.99



Le mois d'après....



Rétic 20% sur frottis

Drépanocytes

Corps de Howell-Jolly

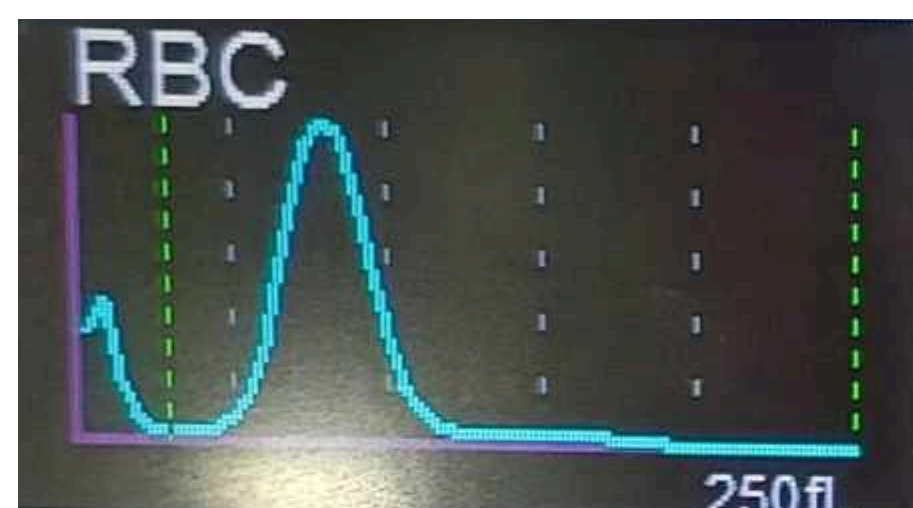
Ret %	32.53	%
Ret	572 000	/mm ³

Cas 4

Nourrisson, MG, âgée de 2 mois, admise pour exploration d'un syndrome anémique avec ictère et SMG

RBC ($10^6/\mu\text{L}$)	2.98
HGB (g/dL)	8.3
HCT (%)	23.7
MCV (VGM) fL	79.5
MCH (TCMH) pg	27.9
MCHC (CCMH) g/dL	35
RDW-CV (%)	13.5
NRBC (%)	1

Rétic (/mm ³)	174 000
RPI (%)	1.5
Micro-R (%)	8.6
Hypo-He (%)	3.1
FRC (%)	0.75
IRF (%)	13.8
LFM (%)	86.2
MFR (%)	9.4
HFR (%)	4.4



TCD	-
Bilan hémolyse	+ (BNC)



Sphérocytose héréditaire HS

HS group (45) / comparator group (1488)

Rule 1: Ret and Ret/IRF ratio

Rule 2: Ret/IRF ratio or MicroR/Hypo-He ratio

Table 2 Hereditary spherocytosis diagnostic tool

Rule		Parameters		
Rule 1	Precondition	Ret $\geq 80,000/\mu\text{l}$ and Ret/IRF > 7.7		
Rule 2	Severity	Trait or mild HS Hb > 12 g/dl Ret/IRF ≥ 19	Moderate HS $8 \text{ g/dl} \leq \text{Hb} \leq 12 \text{ g/dl}$ MicroR $\geq 3.5\%$ and MicroR/Hypo-He ≥ 2.5	Severe HS Hb < 8 g/dl MicroR $\geq 3.5\%$ and MicroR/Hypo-He ≥ 2

Sb 100%; Sp 99.3%; VPP 75%, VPN 100%

SH modérée

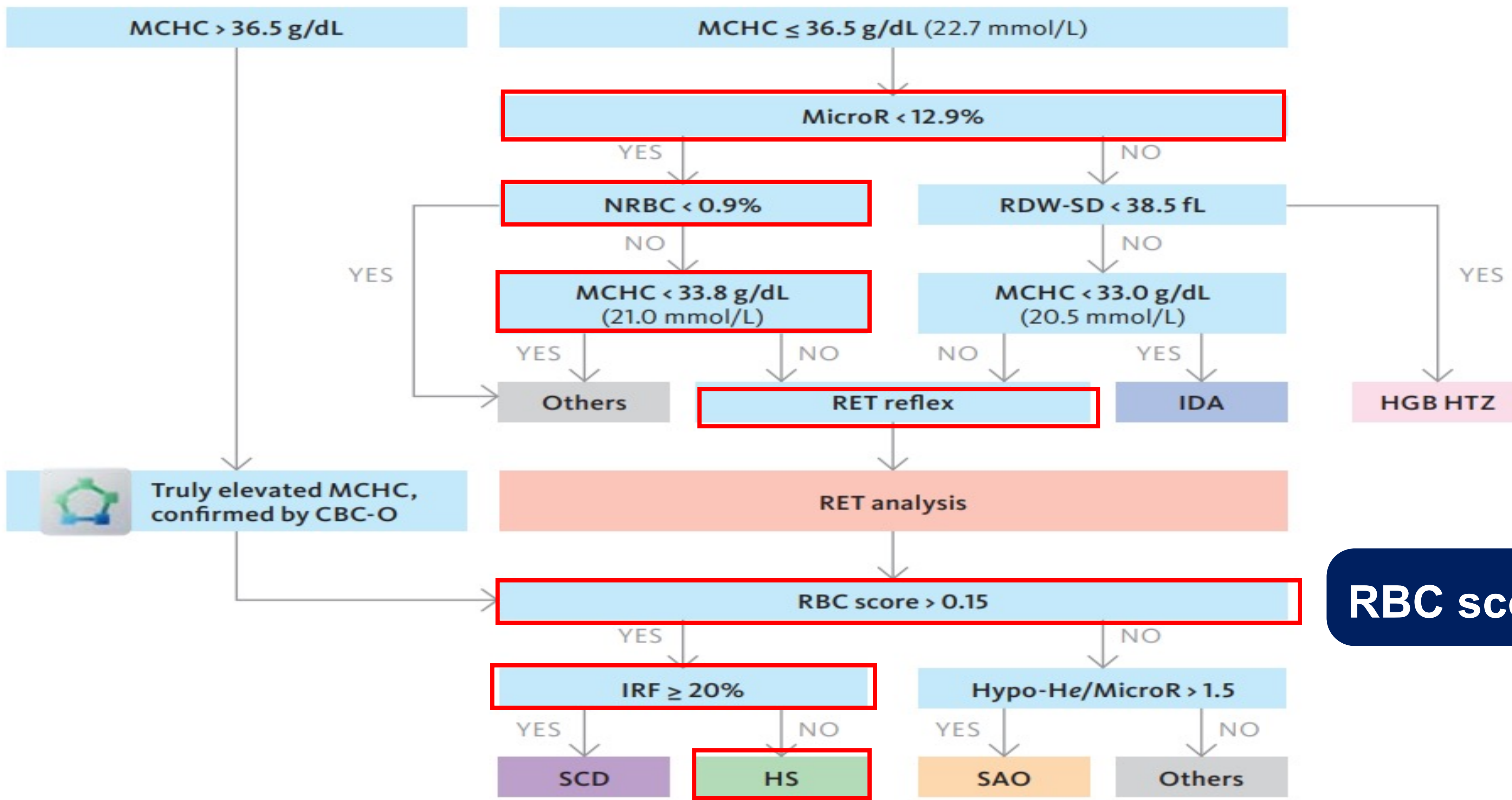
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RET 174 000 /mm³ > 80 000 /mm³
Ret/IRF 12.6 > 7.7

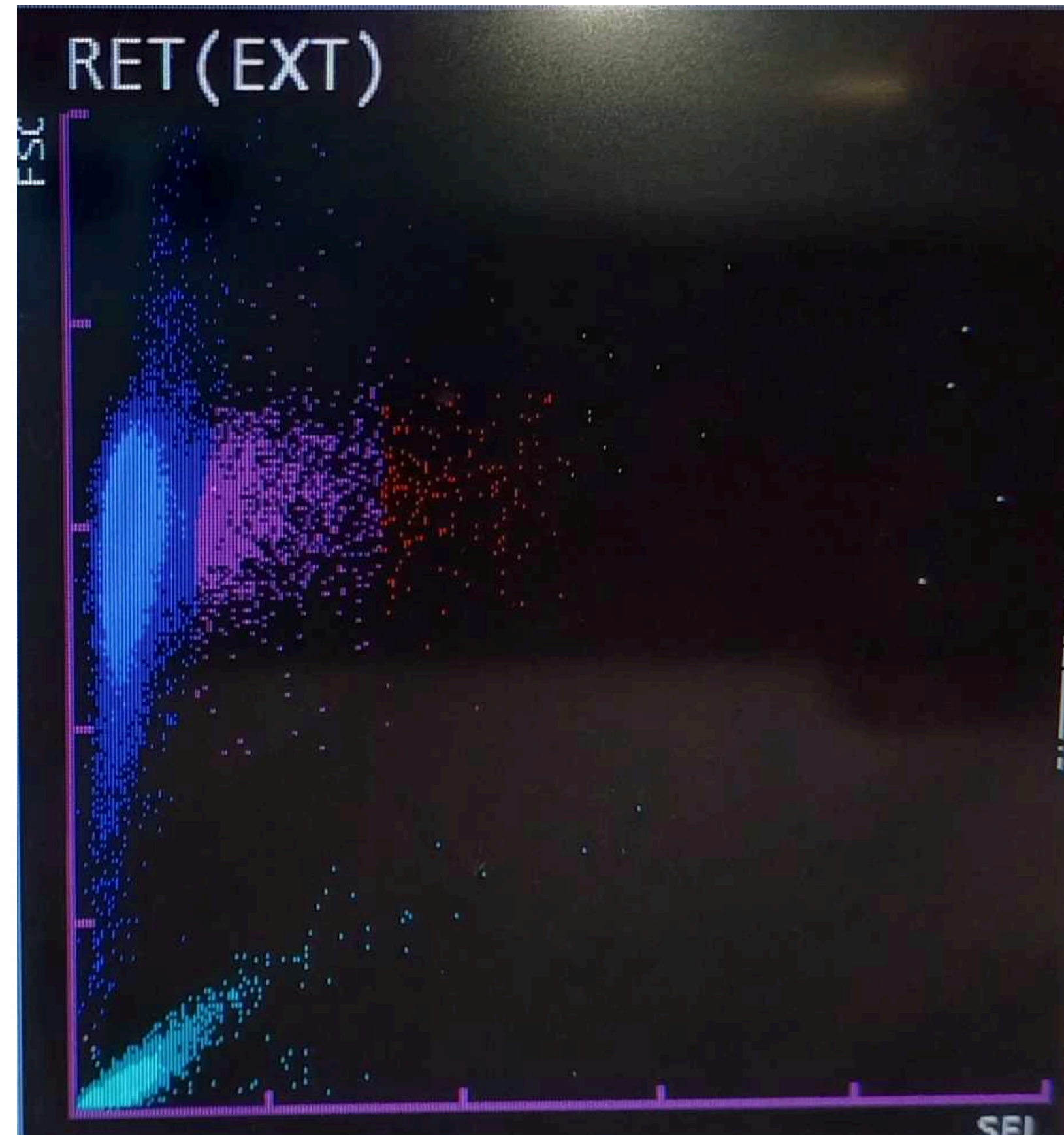
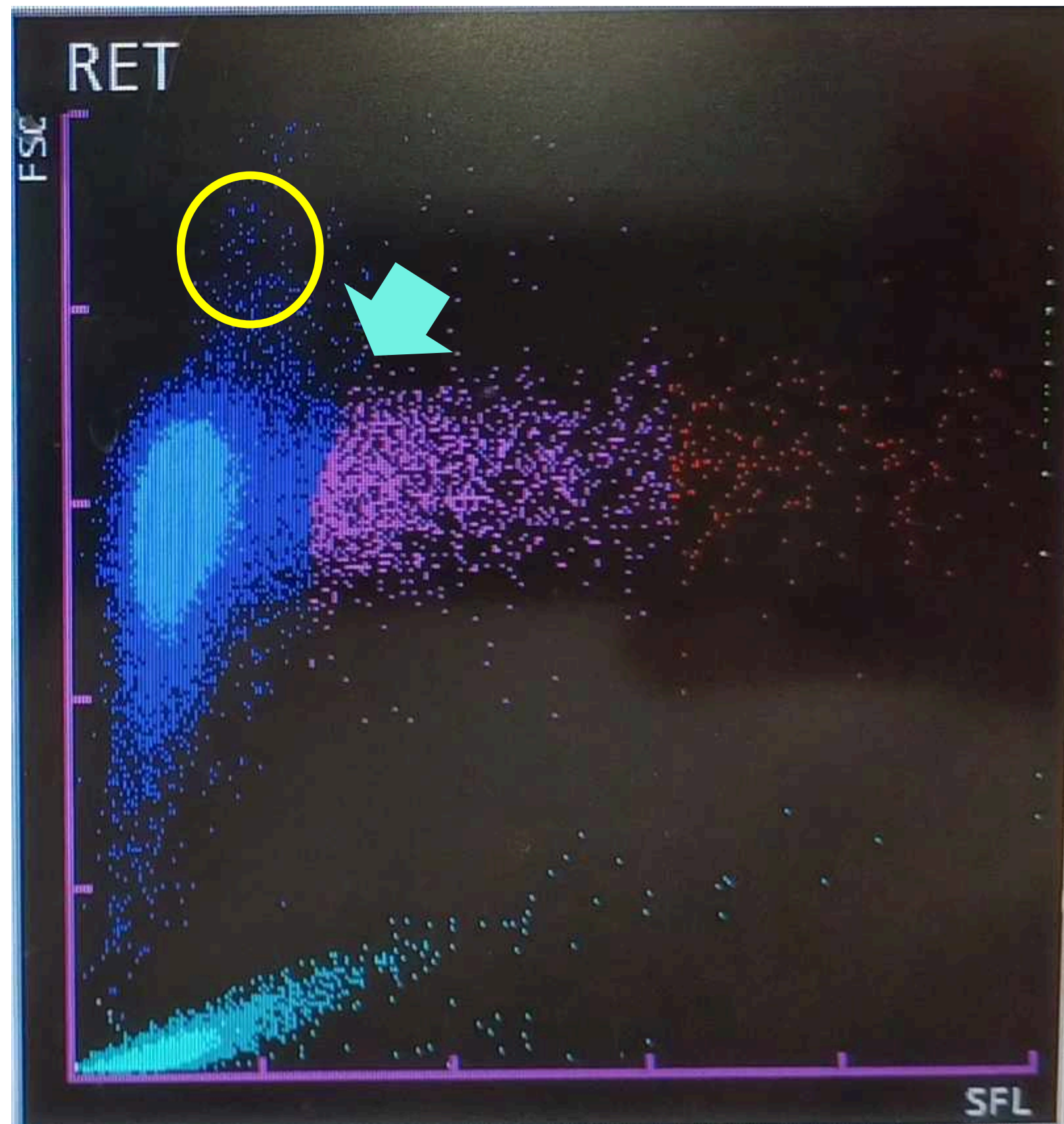
Hb 8.3 g/dL
MicroR 8.6% > 3.5%
MicroR/hypo-He 2.77 > 2.5

RC: ATCD familiaux d'anémie NN



RBC score = 0.64

Cas 4





ICSH guidelines for the laboratory diagnosis of nonimmune hereditary red cell membrane disorders

M.-J. KING*, L. GARÇON†, J. D. HOYER‡, A. IOLASCON§, V. PICARD¶, G. STEWART**, P. BIANCHI††, S.-H. LEE‡‡¹, A. ZANELLA††, FOR THE INTERNATIONAL COUNCIL FOR STANDARDIZATION IN HAEMATOLOGY

Table 1. Criteria for diagnosis of hereditary spherocytosis and general observations

Red cell indices, morphology, and other parameters

- Hemolysis indicators: low or no haptoglobin; raised LDH; hyperbilirubinemia (unconjugated)
- Red cell morphology: **Predominantly spherocytes**. Mild HS shows occasional spherocytes or only during hemolytic crisis. **Mushroom-shaped cells (association with band 3 defects)** (Appendix A). Anisocytosis in patients with severe hemolytic anemia

Red cell indices:

- Hb: variable, often lower than the normal range. May be normal
- **Increased % hyperdense cells** on Advia analyzer [32]
- **MCHC in the high range or increased, reflecting RBC dehydration** [17]
- **Increased delta (MCV-MSCV) value on Beckman Coulter analyzers** [34, 36]
- **Reticulocyte count: increased. Low percentage of immature reticulocyte fraction (increased RET/IRF% ratio) on Sysmex analyzers, especially in mild HS** [35]. **Reduced reticulocyte volume under 100 fL, except in neonates** [37]

% GR

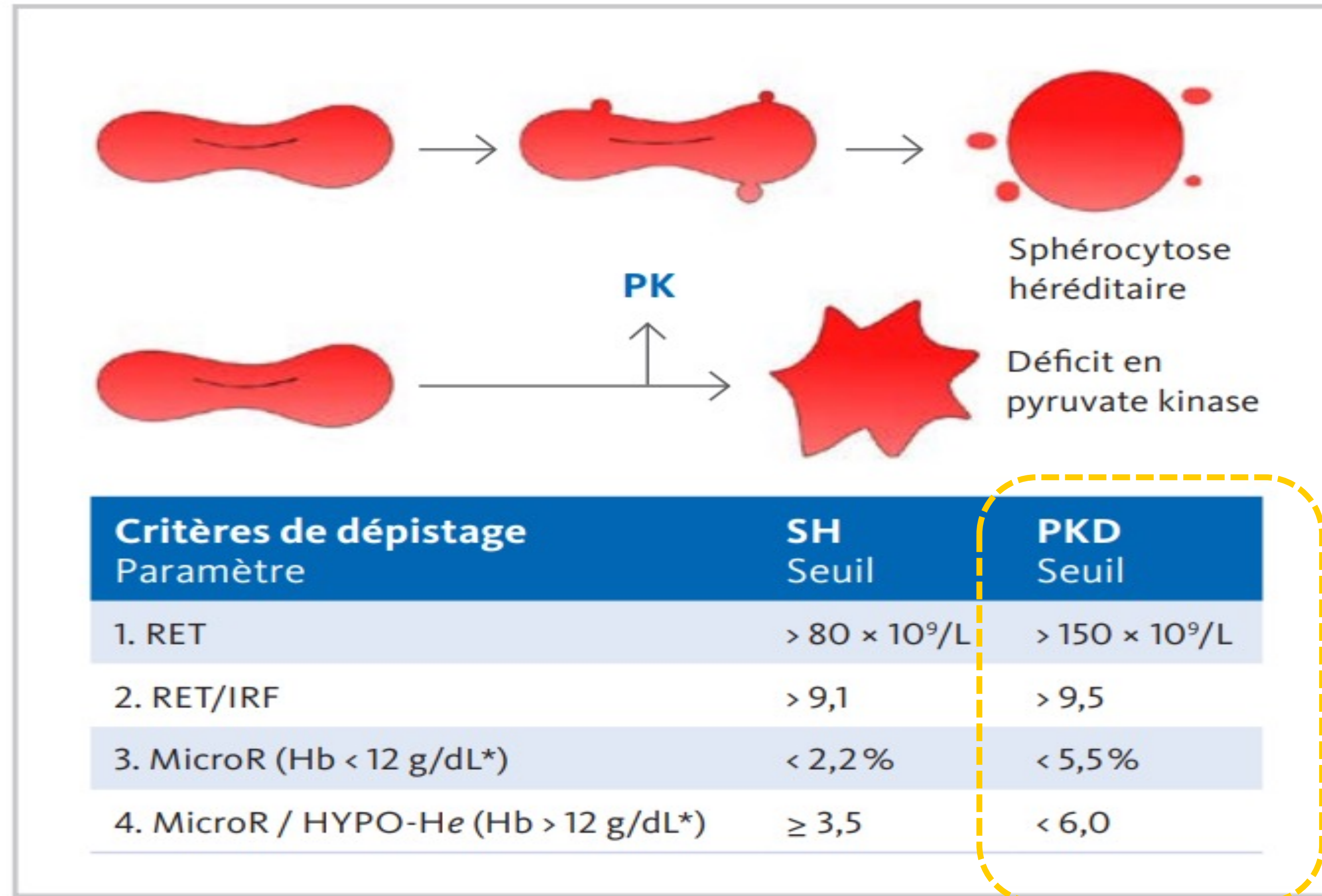
hyperdense
CCMH

MCV-MSCV

RET/IRF

MRV

Déficit en pyruvate kinase



*Les concentrations d'Hb ne sont prises en compte que pour les patients SH

Cas 5

Femme, KZ, âgée de 32 ans, fièvre prolongée, confusion
syndrome anémique d'installation récente (5jours)

WBC ($10^3/\mu\text{L}$)	7.22
RBC ($10^6/\mu\text{L}$)	2.22
RBC-O ($10^6/\mu\text{L}$)	2.17
HGB (g/dL)	6.3
HCT (%)	20
MCV (VGM) fL	90.1
MCH (TCMH) pg	28.4
MCHC (CCMH) g/dL	31.5
RDW-CV (%)	20.4
PLT -I ($10^3/\mu\text{L}$)	10
PLT-O ($10^3/\mu\text{L}$)	14

Ret (%)	18.01
RET (/mm ³)	399 800
RPI	3.6
Micro-R (%)	8.9
Macro-R (%)	5.6
MFV (fL)	92

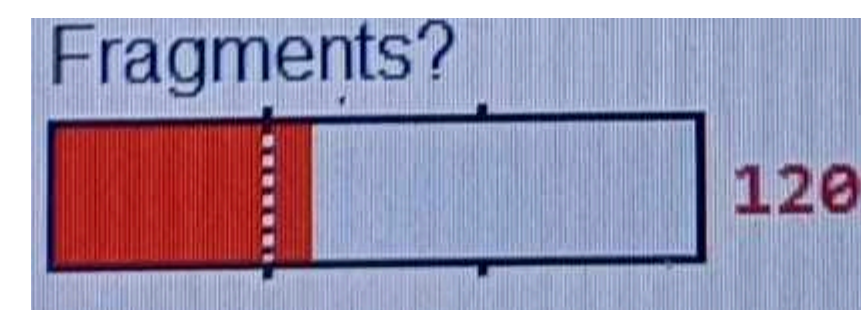
TCD-

hémolyse + (BNC, LDH, haptoglobine)

CRP-

SAM éliminé

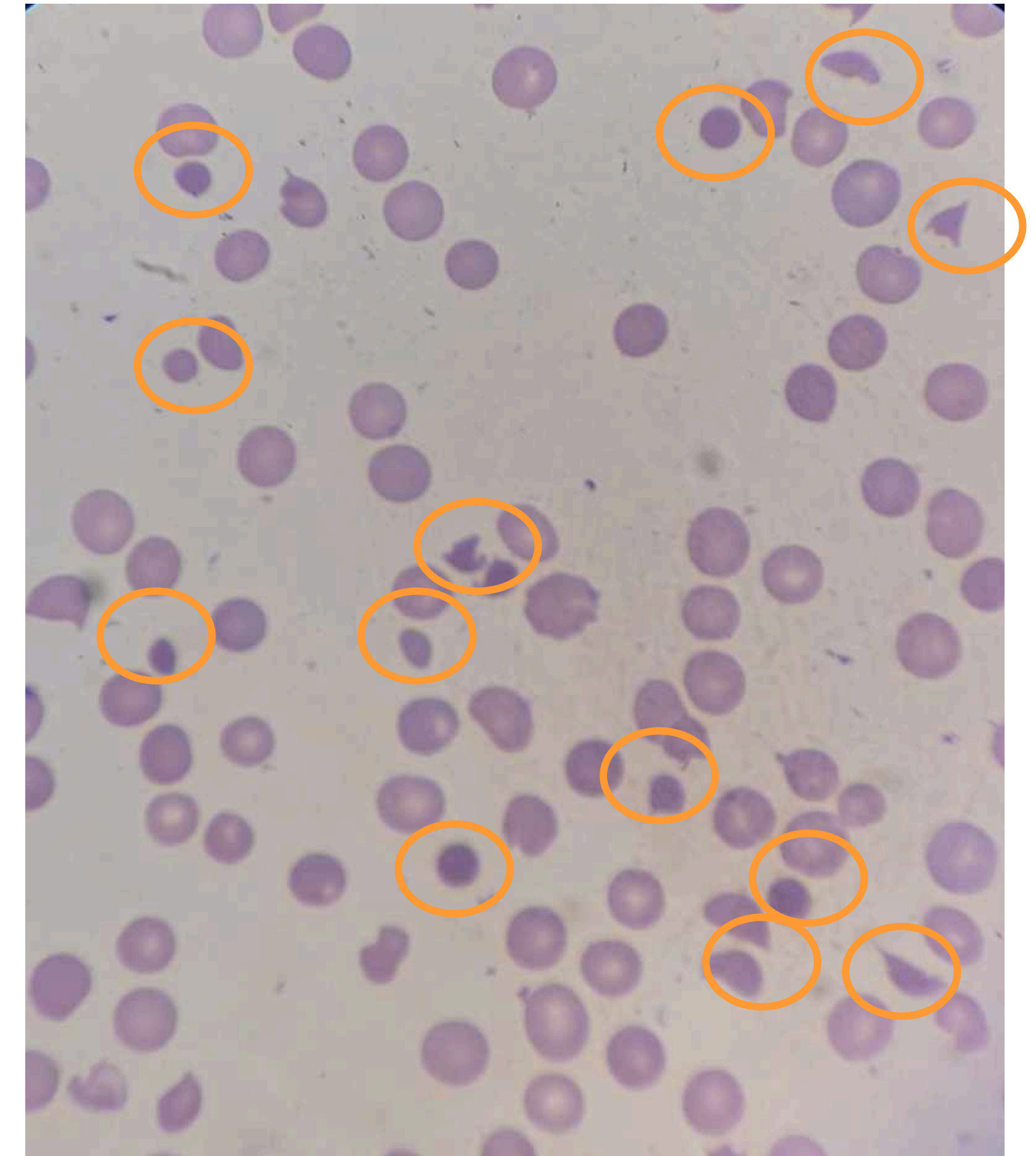
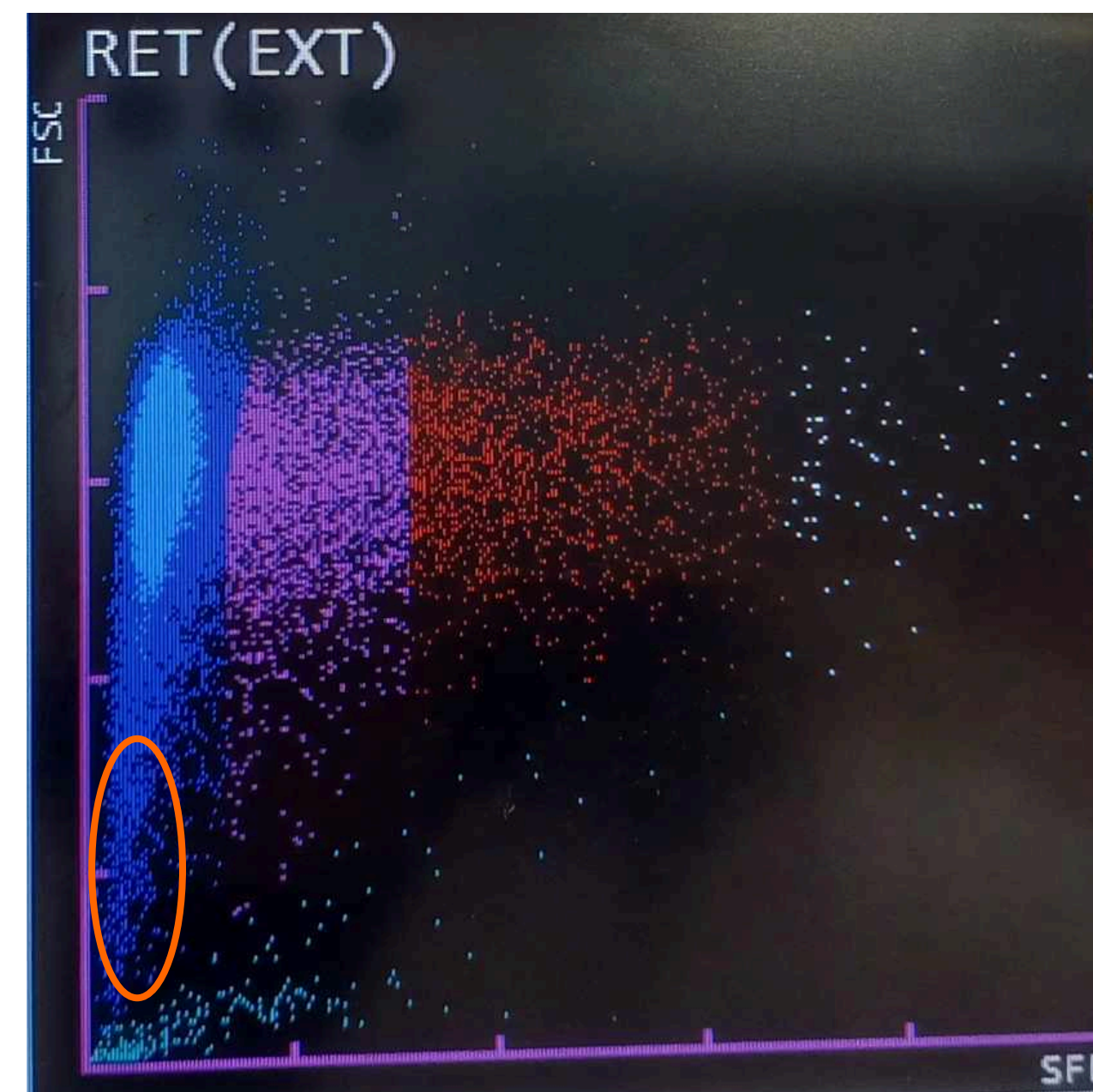
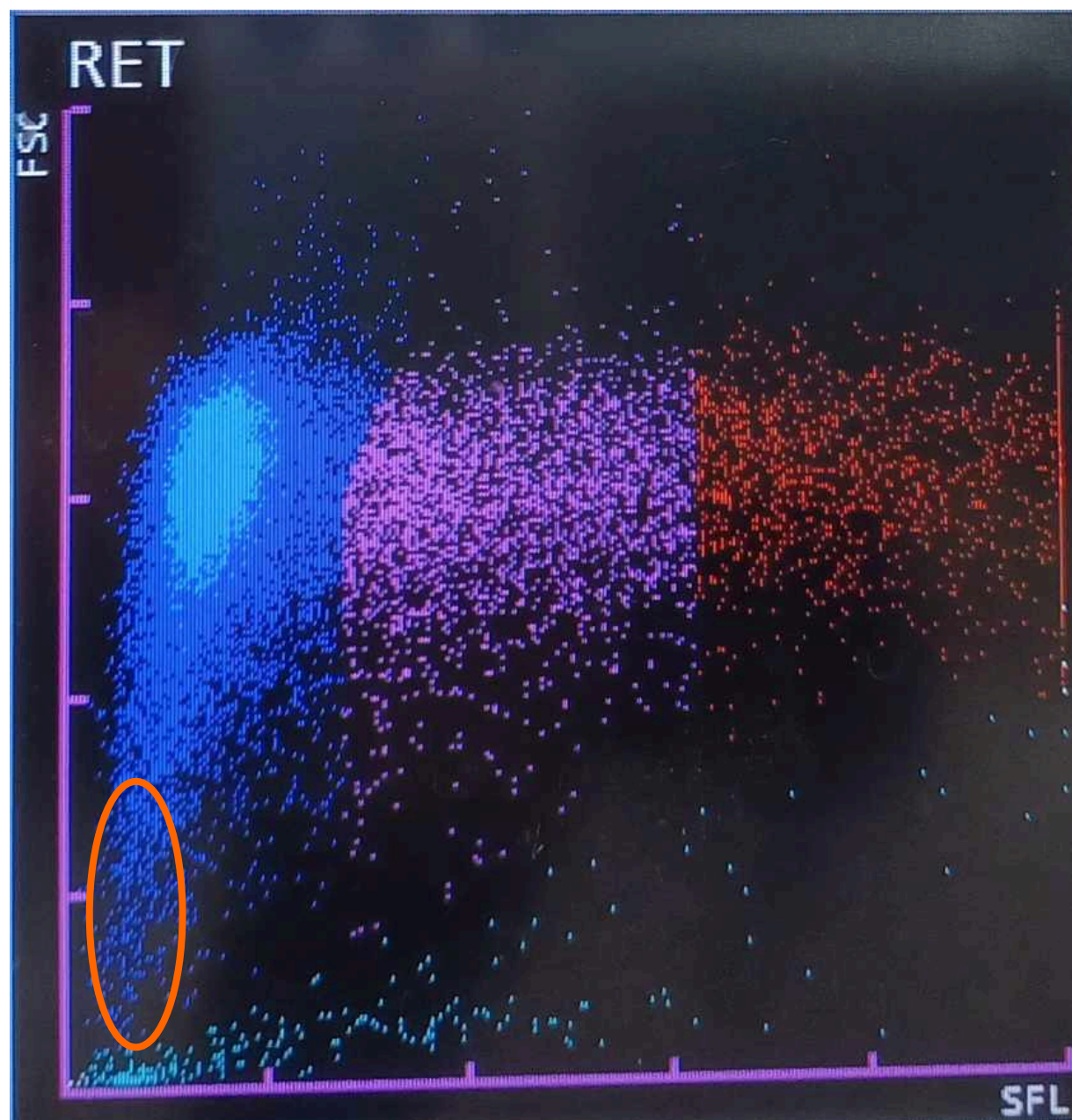
FRC (%)	4.41
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Cas 5

'Alarme FRC' et *schizocytes* sur frottis: « VPN »

FRC (%)	4.41
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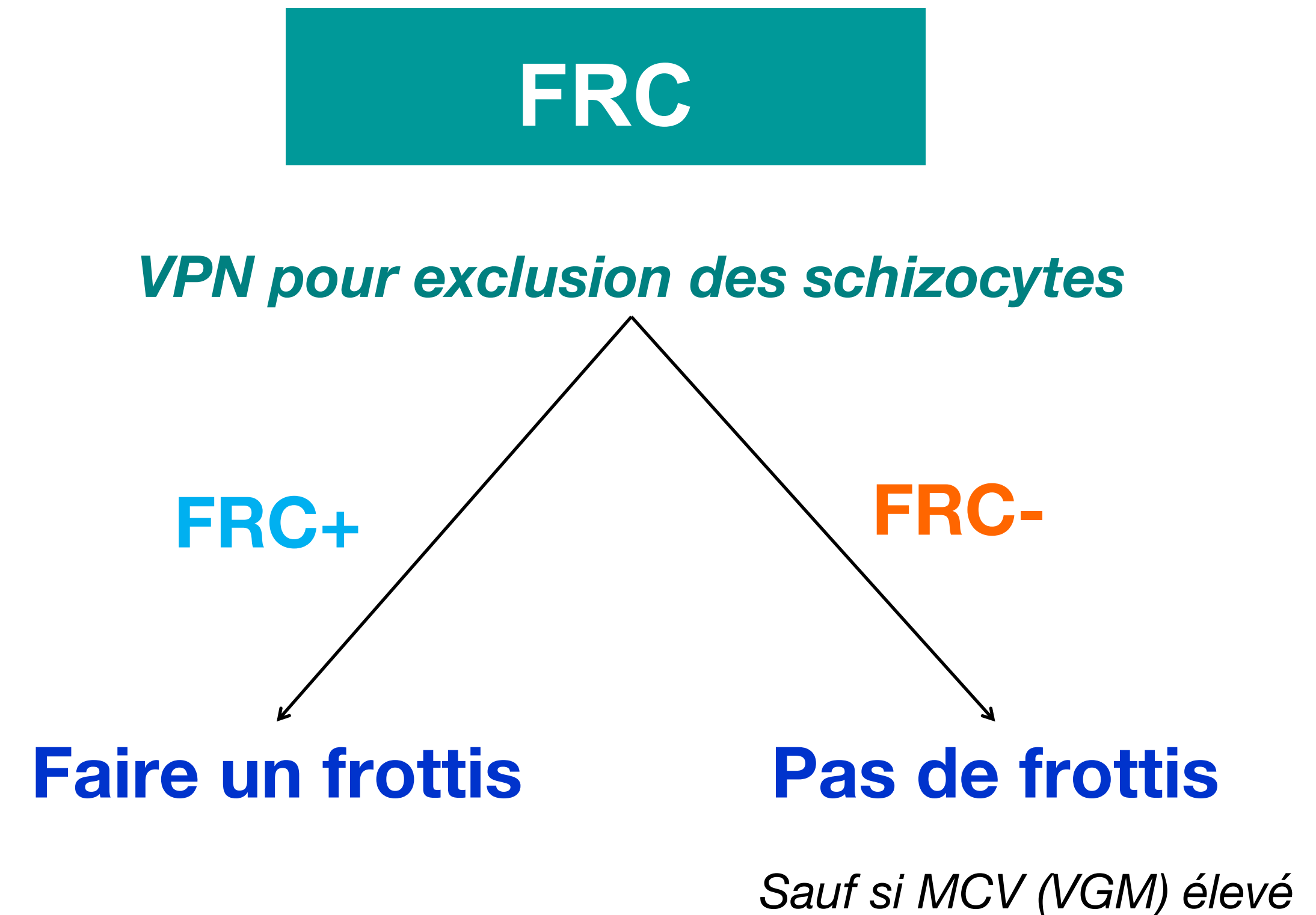


Frottis: 18% de schizocytes

2021 update of the 2012 ICSH Recommendations for identification, diagnostic value, and quantitation of schistocytes: Impact and revisions

In summary, the ICSH Expert Panel, therefore, recommends the following evidence-based statements for automated schistocyte counting:

1. FRC results and flags provided by automated systems are a helpful screening test for assessing schistocytes.
2. The absence of FRCs is a valuable parameter to exclude schistocytes on the blood film, except when the MCV is elevated, which should be checked with the OM.
3. Multicenter studies are required on automated analyzers to generate an FRC reference range and verify intermanufacturer and intermethod comparability.
4. Samples with positive automated FRC count should be reviewed by OM to confirm and count schistocyte
5. Schistocyte preclassification provided by digital morphology analyzers requires confirmation with the OM, following the general ICSH Recommendations on digital morphology analyzers.⁷⁴



FRC et MAT

Exclusion de MAT :

- Transplantation hépatique
- Greffe de moelle osseuse

Un seuil FCR 1.2%

Sb 90%, Sp 96%, VPP 90%; VPN 90%

ATTENTION

- 3h après le prélèvement si conservation à température ambiante ou 8h si conservation à +4°C.
- MCV (VGM) élevé...risque de **FN FRC**
- Hypochromie modérée interfère avec% FRC.

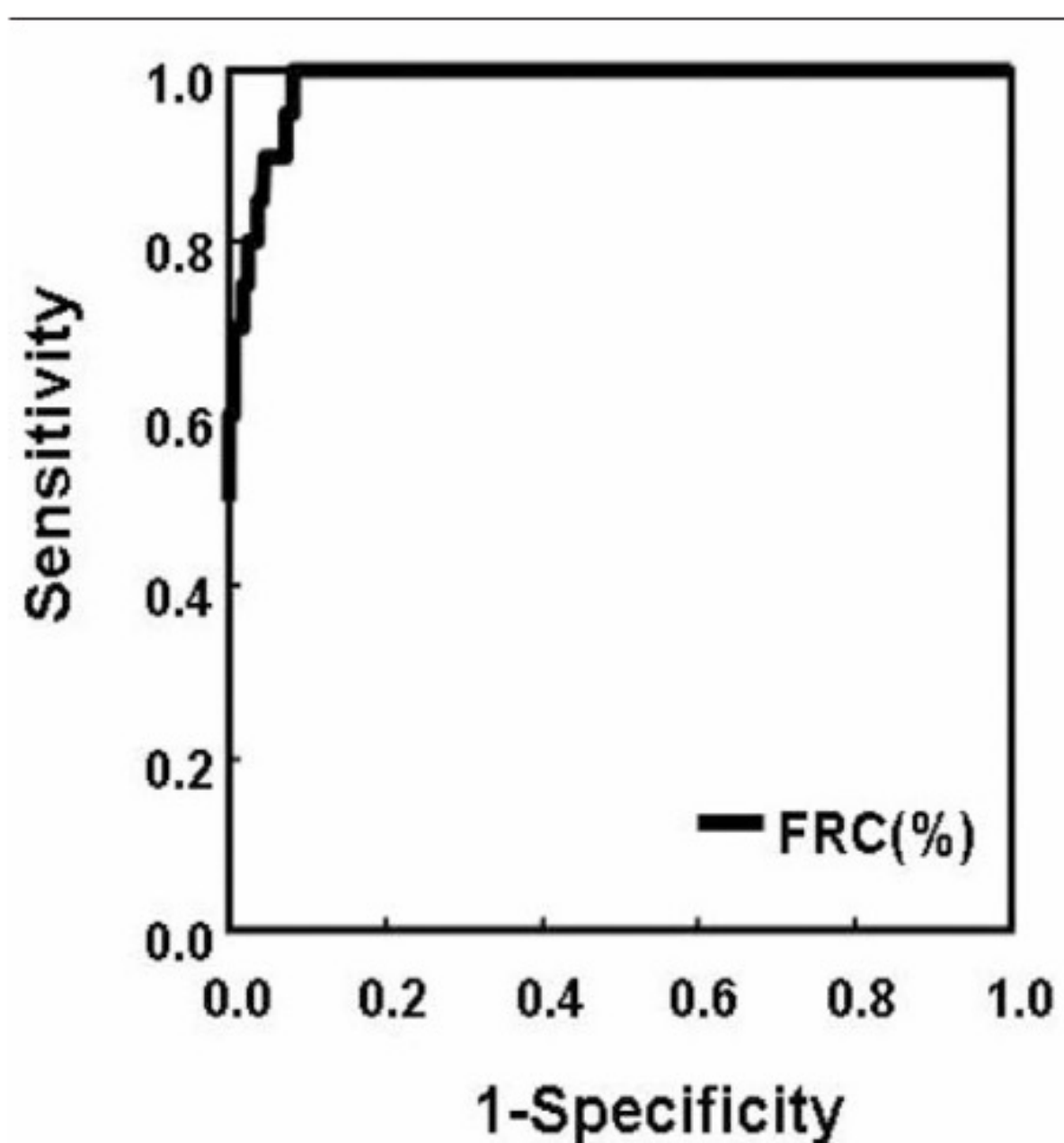
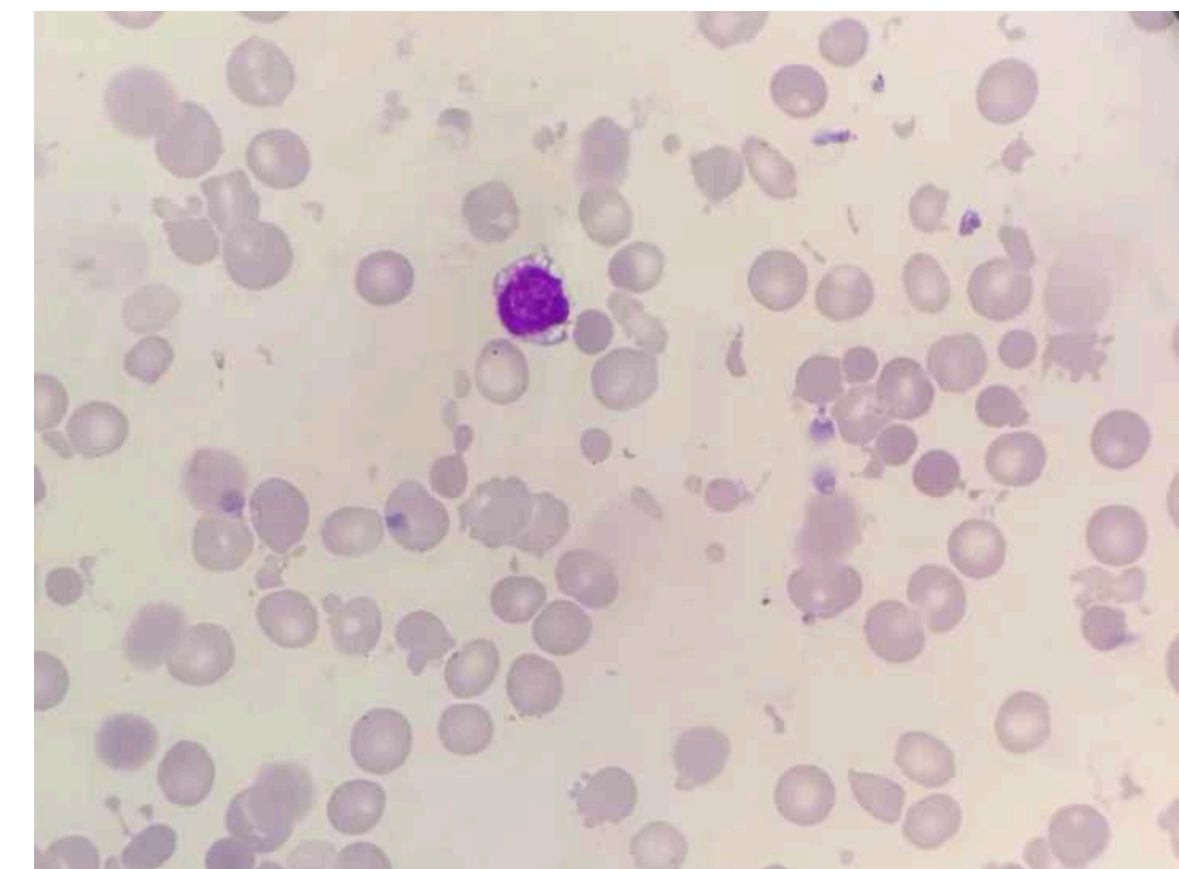


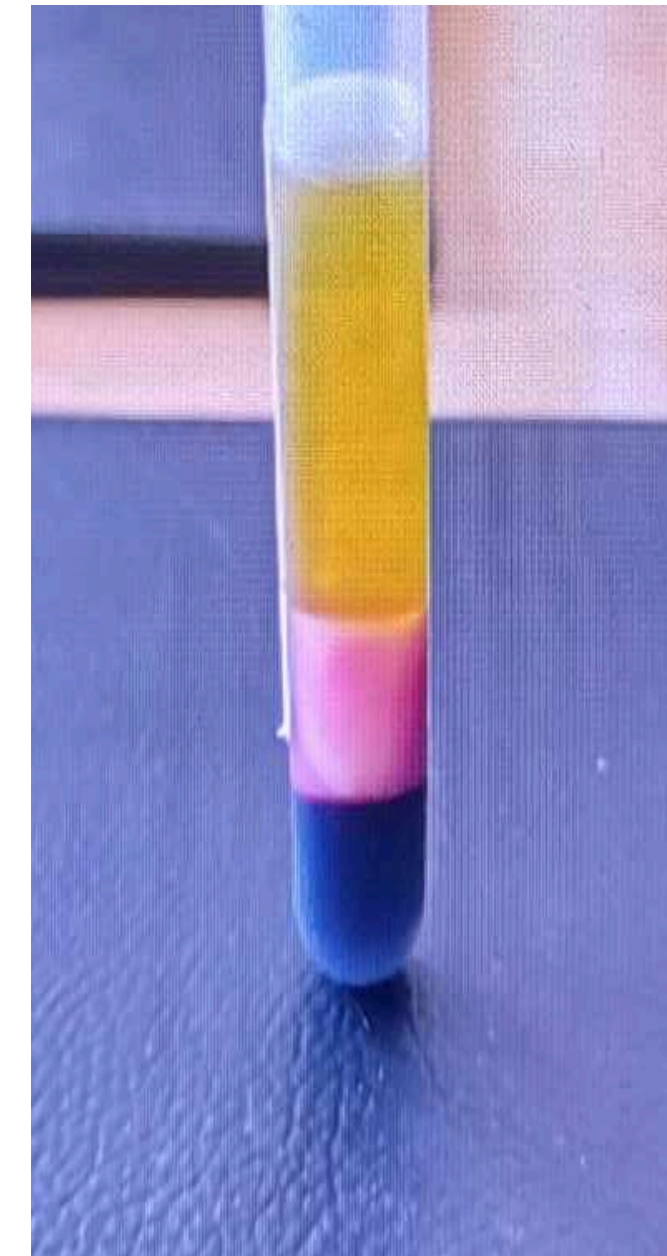
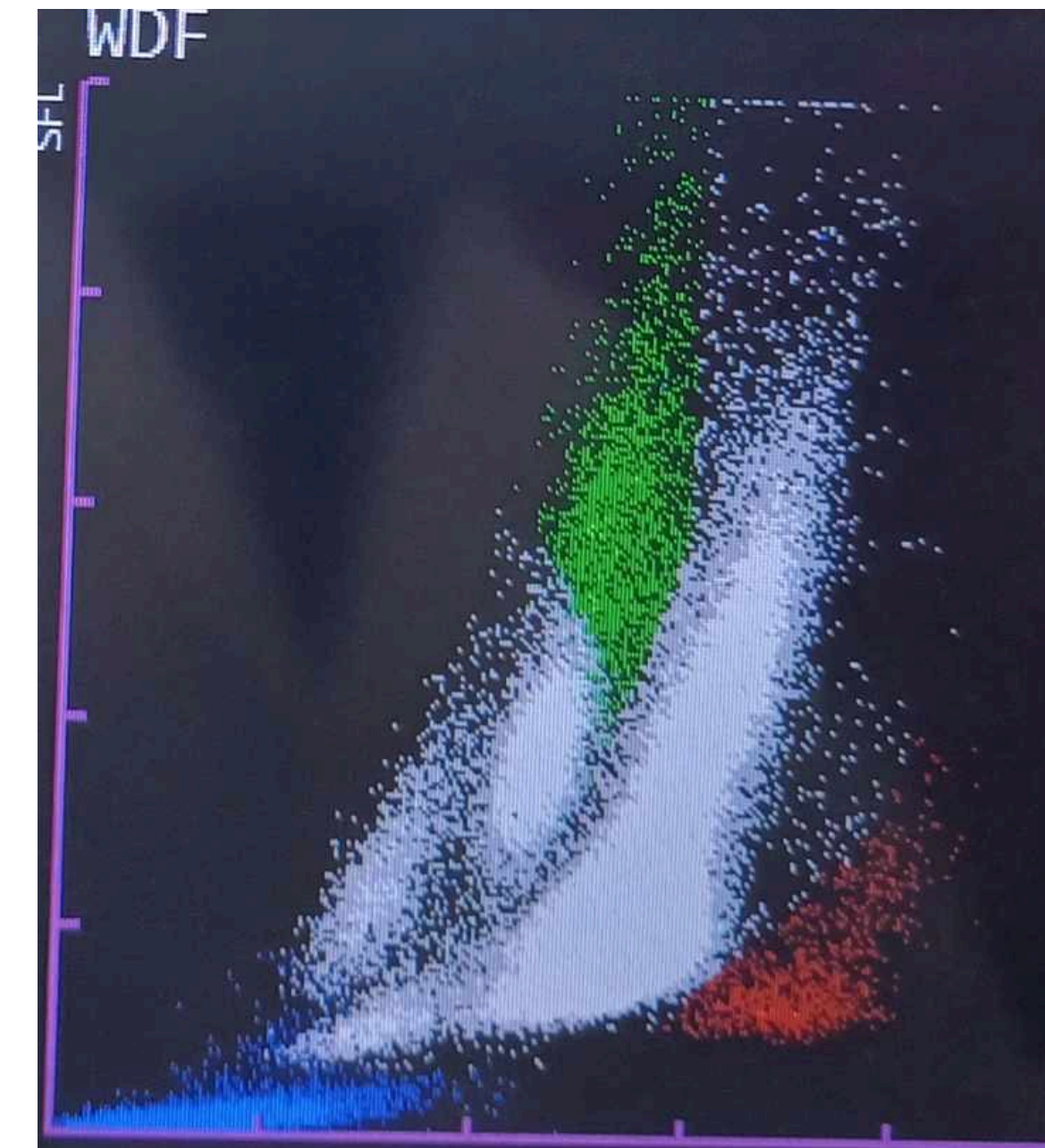
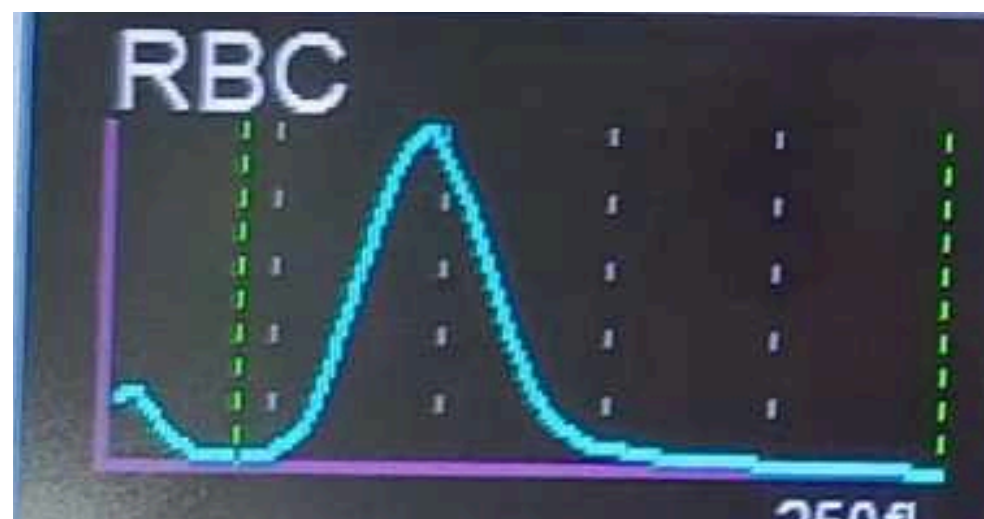
Figure 3. A receiver operating characteristic analysis of fragmented red cell (FRC) level for the diagnosis of thrombotic microangiopathy.



Cas 6

Homme, MB, âgé de 37ans, SMG, syndrome anémique

WBC ($10^3/\mu\text{L}$)	450
RBC ($10^6/\mu\text{L}$)	2.79
HGB (g/dL)	8.2
MCV (VGM) fL	95.3
MCH (TCMH) pg	29
MCHC (CCMH) g/dL	30.5
PLT -I ($10^3/\mu\text{L}$)	255



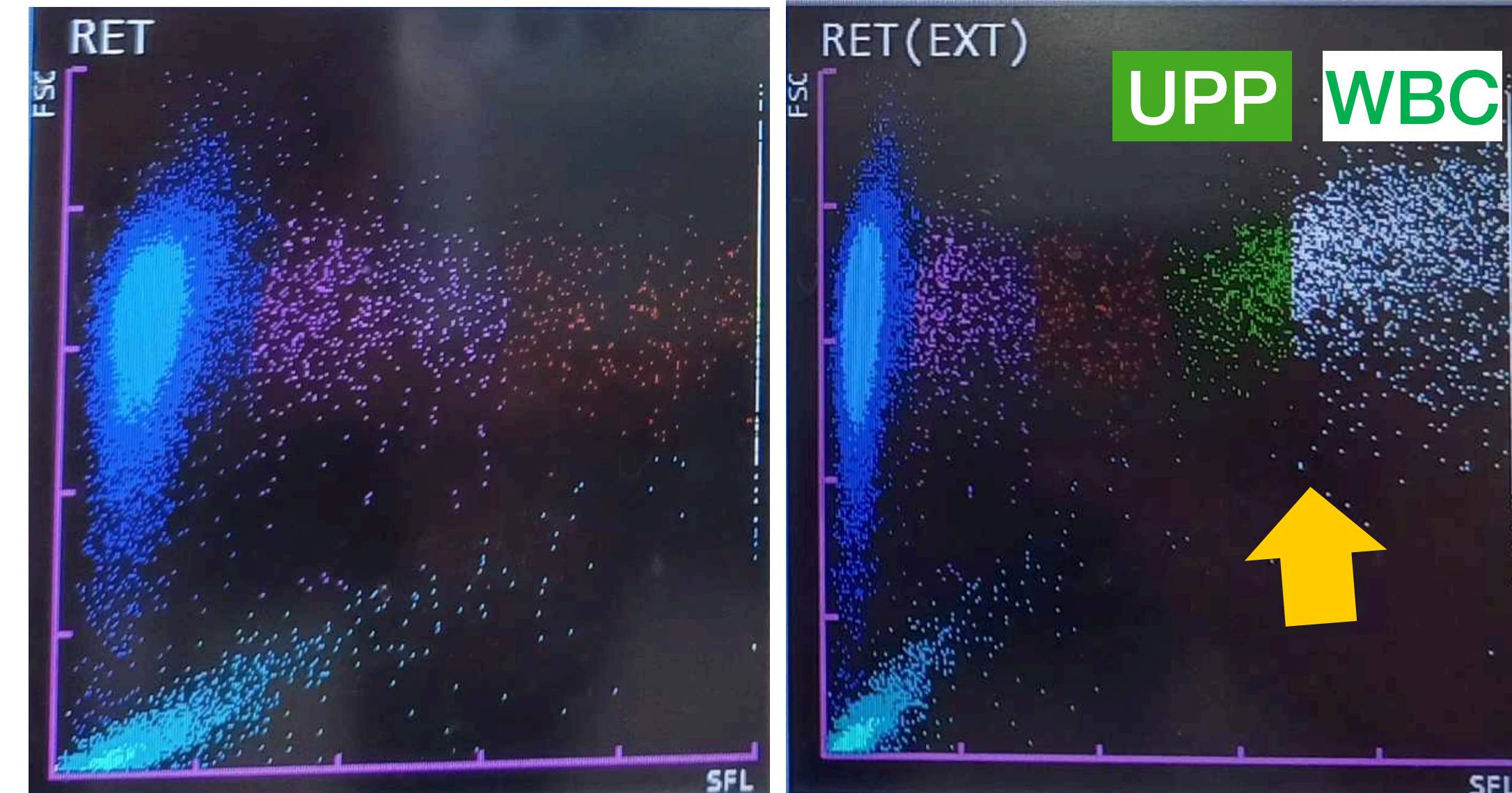
LMC

Cas 6

Homme, MB, âgé de 37ans, SMG, syndrome anémique

WBC (10 ³ /μL)	450
RBC (10 ⁶ /μL)	2.79
HGB (g/dL)	8.2
MCV (VGM) fL	95.3
MCH (TCMH) pg	29
MCHC (CCMH) g/dL	30.5
PLT -I (10 ³ /μL)	255

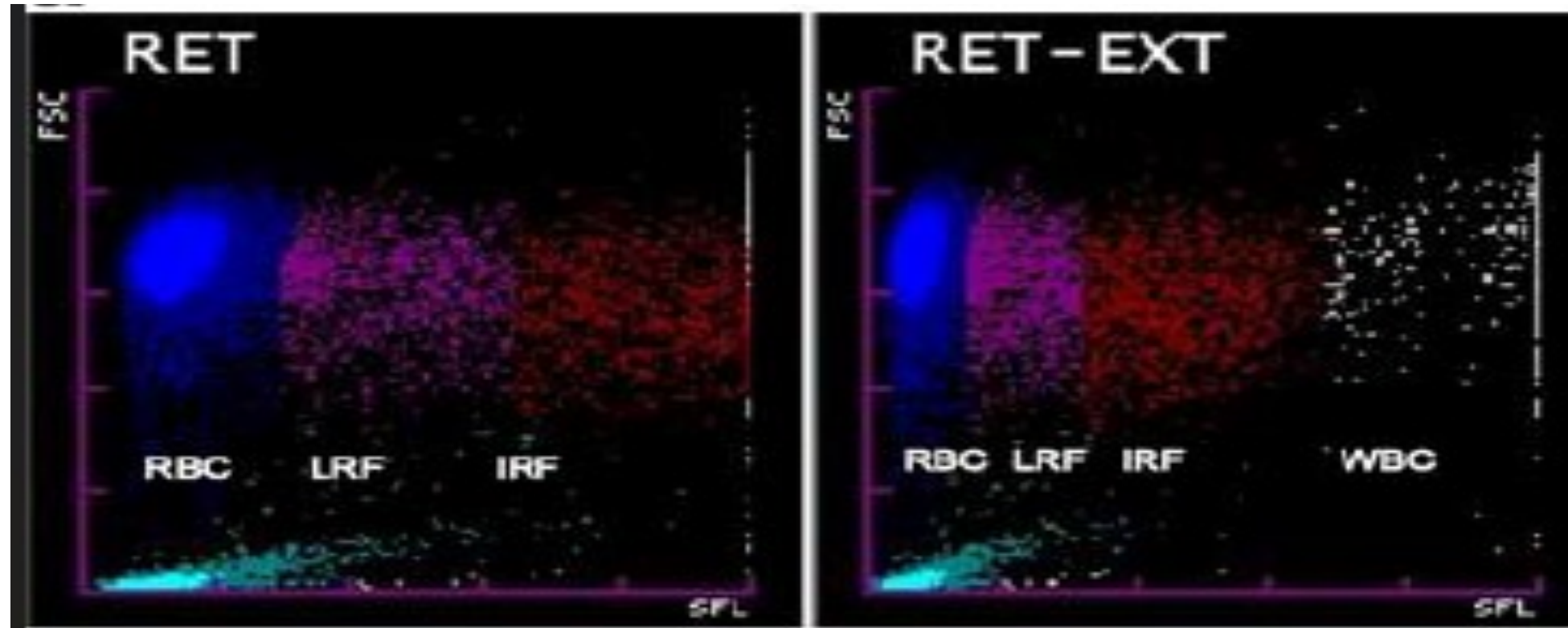
Ret (%)	3.8
Ret (/mm ³)	106 020
IRF (%)	30%
RET-UPP	389



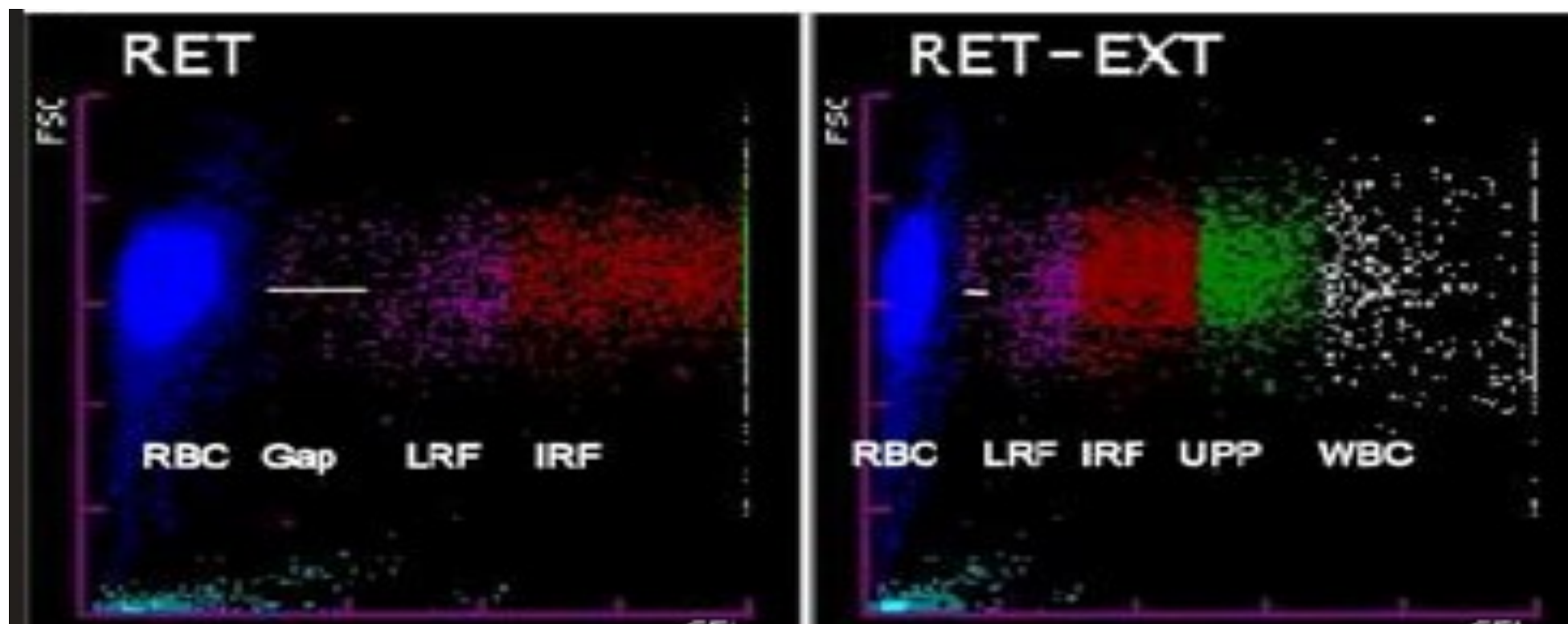
Anemia
RET Abn Scg

Rétic sur frottis: 1.6%
(=44 640/mm³)

Ret normal Scattergramme



Ret abnormal Scattergramme



Écart entre GR et RET
UPP > 100
IFR augmenté

« **pseudoréticulocytose** »



Dilution pour avoir UPP < 100 et disparition
de l'alarme « Ret Abn Scg »

Laboratoire du Centre de Maternité et de Néonatalogie-Monastir

