Cellular Biomarkers in Patient Blood Management

ASPA 2024, Kuching, Sarawak, Malaysia,

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ASPA 2024

CONFLICT OF INTEREST

Prof. Hönemann was paid in the last 3 years
salary for scientific and congress presentation from
following companies:

Draeger, GmbH&CoKG, Lübeck, Germany
Vifor Pharma GmbH, München, Germany
Sedana medical GmbH, Geretsried-Gelting, Germany
Ärztekammer Niedersachsen, Hannover, Germany
WIVIM e.V. , Bremen, Germany
DGAI e.V. Nürnberg, Germany
Sysmex Deutschland GmbH, Germany

PREOPERATIVE ANEMIA

Prevalence

ANEMIA IN PAEDIATRIC PATIENTS IS COMMON, A WIDESPREAD DISEASE

= Anemic   Non = Anemic

Pediatric surgery
> 30 %
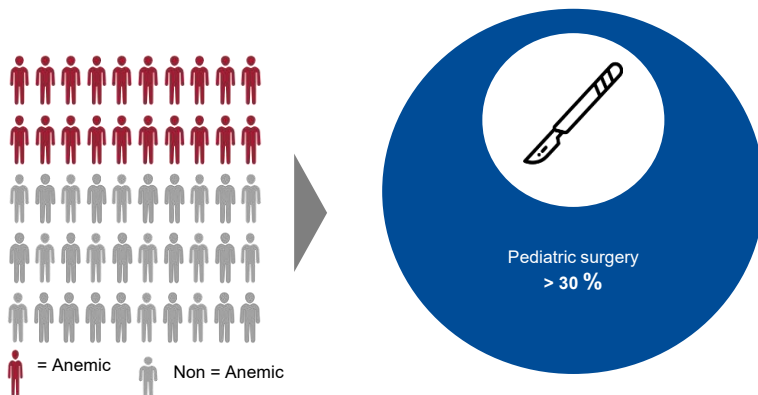
Pediatric Anesthesia

RESEARCH REPORT

The association between preoperative anemia and postoperative morbidity in pediatric surgical patients: A secondary analysis of a prospective observational cohort study

Heidi M. Meyer, Alexandra Torborg, Larissa Cronje, Jennifer Thomas, Anisa Bhettay, Johan Diedericks, Celeste Cilliers, Hyla Kluyts, Busisiwe Mrara, Mandisa Kalipa, Bruce Biccard ... See all authors

First published: 10 April 2020 | https://doi.org/10.1111/pan.13872 | Citations: 12

See SAPSOS Investigators in Supporting Information.

ANEMIA IN PAEDIATRIC PATIENTS

MOST COMMON CAUSES?

Anemia in infants and children (table by age: Newborn (0–30 days), Infant (0–1 year), Toddler (2–3 years), Preschool (4–5 years), Child (6–9 years), Preteen (10–12 years), Teenager (13–18 years); disorders: Membrane defects, Abnormalities of metabolism, Unstable hemoglobins, Sideroblastic anemia, α-Thalassemia, β-Thalassemia, Sickle cell disease, Congenital dyserythropoietic anemia, Diamond-Blackfan anemia, Fanconi anemia, Hemolytic uremic syndrome, Thrombotic thrombocytopenic purpura, Disseminated intravascular coagulation, Hemorrhage, Chronic inflammation, Malignancies, Neonatal alloimmune hemolytic disease, Primary autoimmune hemolytic anemia, Secondary autoimmune hemolytic anemia, Aplastic anemia, Iron deficiency, B12 deficiency, Folate deficiency)

CAUSES OF PREOPERATIVE ANEMIA IN PAEDIATRIC SURGERY

MOST COMMON CAUSE: LACK OF IRON[1]

ca. 40 – 50%
Lack of Iron
Iron deficiency anemia (IDA)

ca. 5%
Rare forms Unclear anemia, deficiency in vitamin B12 / folic acid, genetic mutation

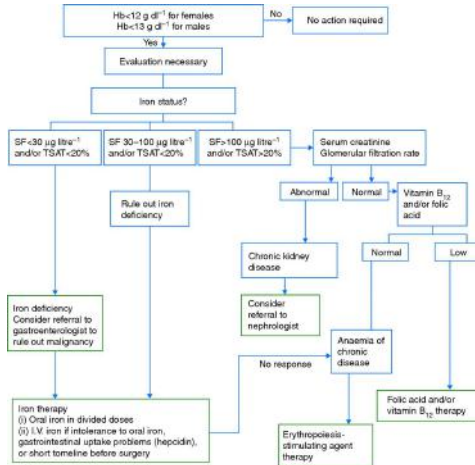| ca. 40% |
| --- |
| Lack of free iron |
| **Anemia of chronic Diseases (ACD)** |

Preoperative Anemia
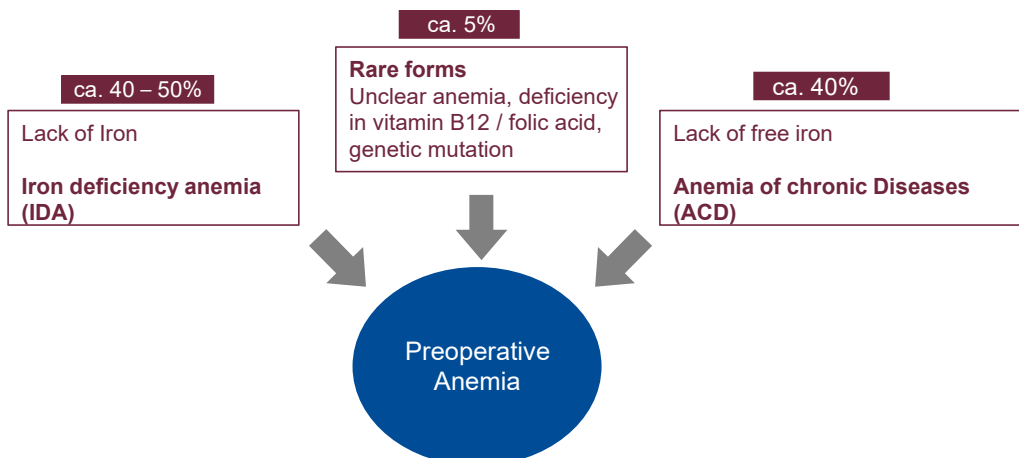
1. Meybohm P et al. Chirurg 2016; 87: 40–46.

Modifiziert nach Meybohm P et al. 2016.

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PROPOSED ALGORITHM FOR THE DETECTION, EVALUATION, AND MANAGEMENT OF PREOPERATIVE ANAEMIA. SF, SERUM FERRITIN; ...
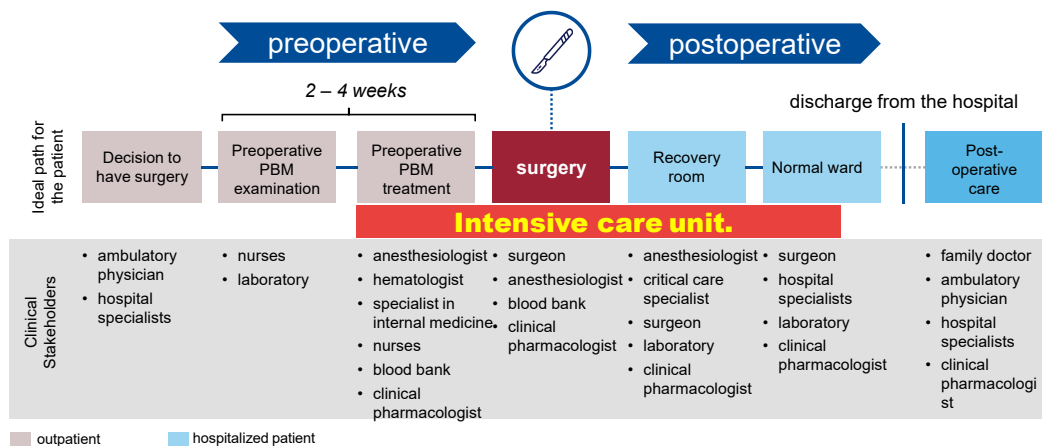
OXFORD
UNIVERSITY PRESS

PBM IS AN INTERDISCIPLINARY AND MULTIMODAL APPROACH – WE NEED AN EARLY DIAGNOSIS OF THE CAUSE OF ANEMIA

Ideal path for the patient	Decision to have surgery	Preoperative PBM examination	Preoperative PBM treatment	surgery	Recovery room	Normal ward	Post-operative care

2 – 4 weeks · preoperative / postoperative · discharge from the hospital · **Intensive care unit.**

Clinical Stakeholders							
• ambulatory physician • hospital specialists	• nurses • laboratory	• anesthesiologist • hematologist • specialist in internal medicine • nurses • blood bank • clinical pharmacologist	• surgeon • anesthesiologist • blood bank • clinical pharmacologist	• anesthesiologist • critical care specialist • surgeon • laboratory • clinical pharmacologist	• surgeon • hospital specialists • laboratory • clinical pharmacologist		• family doctor • ambulatory physician • hospital specialists • clinical pharmacologist

■ outpatient ■ hospitalized patient

1. EU Commission - Supporting Patient Blood Management (PBM) in the EU - A Practical Implementation Guide for Hospitals

Priv. Doz. Dr. med. Christian Hönemann, Marienhospital Vechta und Universitätsklinikum Münster

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Technology XN-1000: Immunofluorescenc Flow Cytometry

Flow cytometry runs on the principles of light scattering, excitation and emission. Fluorescently tagged cell components get excited when they pass through a laser beam, producing lights of different wavelengths. The fluorescence is used to analyze cellular properties.
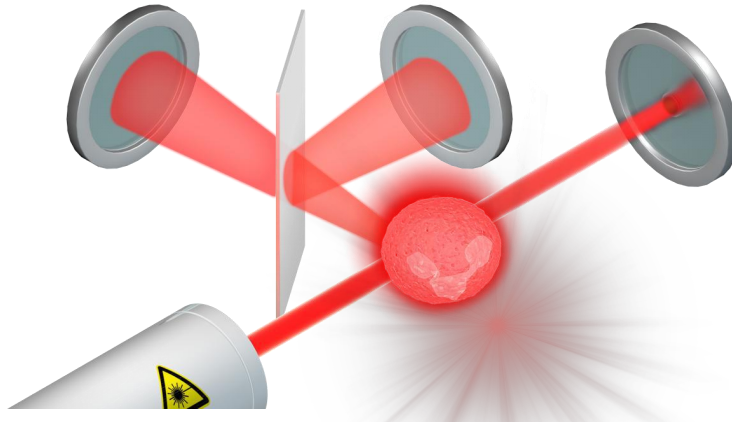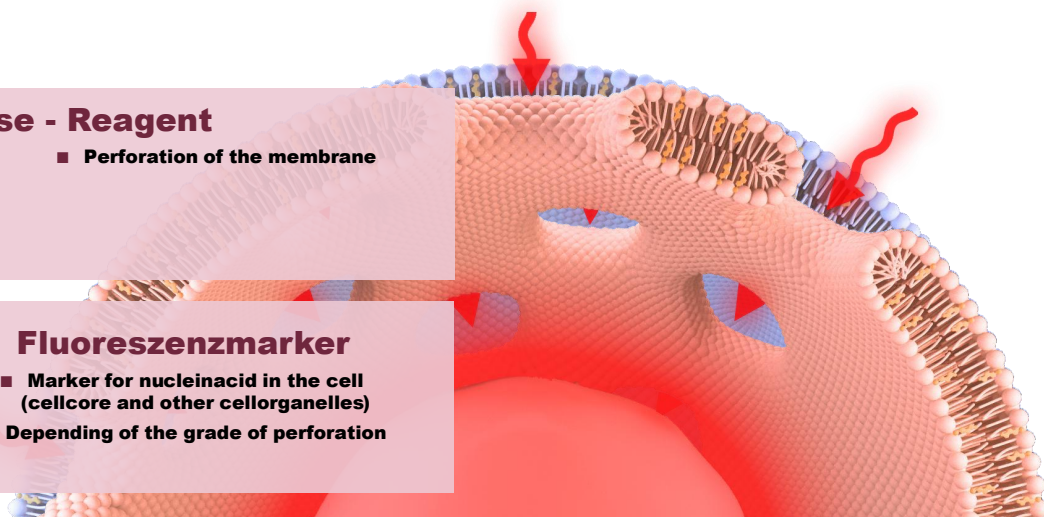
Basic of Sysmex XN technology
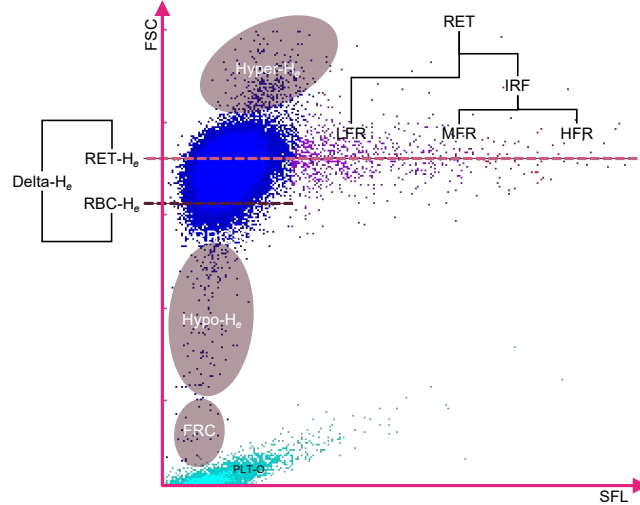
Lyse - Reagent
- **Perforation of the membrane**

Fluoreszenzmarker
- **Marker for nucleinacid in the cell (cellcore and other cellorganelles)**
- **Depending of the grade of perforation**

RETICULOCUTE RET-PARAMETER OF THE XN-SERIES

SYSMEX RETICULOZYTE PARAMETER

Immunofluorescence flow cytometry
Retikulocyte, Reticulocyte - maturity parameters

- **RET-H$_e$ →**   **retikulocyte-hemoglobin equivalent**

- **RBC-H$_e$ →**   **erythrocyten-hemoglobin-equivalent (MCH)**

- **Delta-H$_e$ →**   **difference of hemoglobinisation of immature and mature erythrozyte**

  (RET-H$_e$ minus RBC-H$_e$)

- **Hypo-H$_e$ →**   **ammount of hypochrome erythrocytes**

DIRECT IRON AVAILABILITY FOR ERYTHROPOESIS = RET-H$_E$
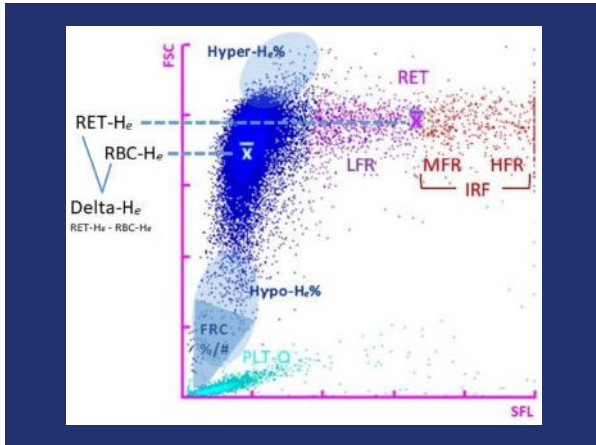
✓ **Reference intervall**

   28,4 – 34,4 pg[6]

■ **Iron deficiency detected even in acute phase reaction**

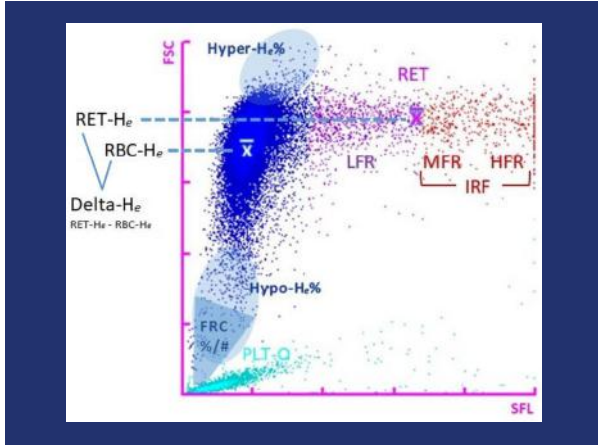■ **RET-H$_e$ can indicate the therapeutic success of an iron dose after just two days**

(6) Park *et al.* 2015

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ACUTE PHASE REACTION – CYTOKINE – HEPCIDINE – IRON DEFICIENCY
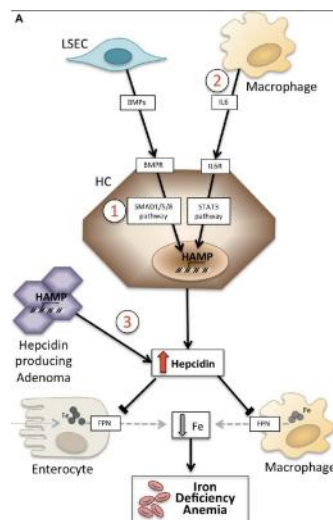
Seite 14

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EARLY MARKER FOR BACTERIAL INFECTION IS DELTA-H$_E$

✓ **Referenc intervall**

1,7 – 4,4 pg[6]

- **RET-H$_e$ – RBC-H$_e$ = Delta-H$_e$**
  (Difference of hemoglobin in immature and mature erythrocytes)

- **Indirect measurement of Monozyten-activity and production of hepcidine in Anemia of chronic disease**
- **Marker of acute infection with short reaction time**

(6) Park *et al.* 2015

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RET-HE FOR THE EVALUATION OF QUALITY AND QUANTITY ERYTHROPOIESIS - NEPHROLOGY

Urrechaga E et al. (2013):

Erythrocyte and reticulocyte indices in the assessment of erythropoiesis activity and iron availability.

Key message:

» Detection of iron deficiency anemia in patients with CKD

» Detection of IDA :
Cut-Off RET-He:
< 29,8pg: AUC 0,935, sensitivity 90,7%,
specifity 83,1%;
240 Pat with CKD

Quelle: Int J Lab Hematol 35: 144-149

PARAMETERS FOR THE DIAGNOSIS OF ANAEMIA

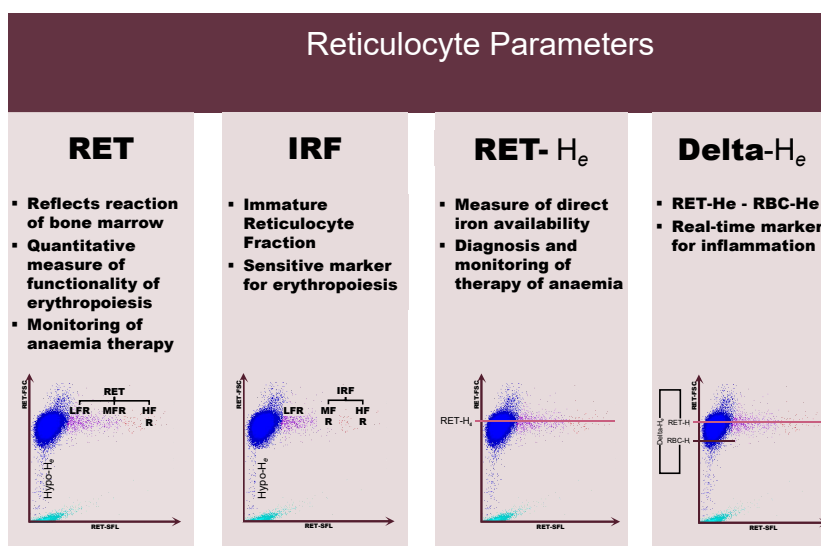**Most biochemical markers are influenced by acute phase reaction**

- **Serum iron**
- **Transferrin**
- **Ferritin**

Clinical interpretation in terms of iron deficiency very often impaired

- **Reticulocyte parameters improve the diagnosis and treatment of functional iron deficiency**
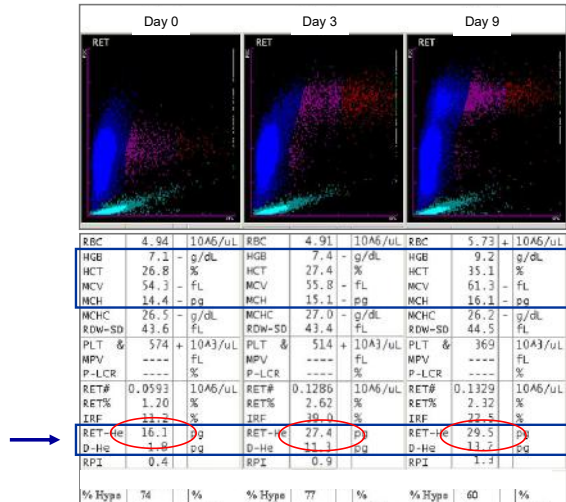
1

ANAEMIA: RETICULOCYTE PARAMETERS

Reticulocyte Parameters			
RET	**IRF**	**RET-** H_e	**Delta-** H_e
• Reflects reaction of bone marrow • Quantitative measure of functionality of erythropoiesis • Monitoring of anaemia therapy	• Immature Reticulocyte Fraction • Sensitive marker for erythropoiesis	• Measure of direct iron availability • Diagnosis and monitoring of therapy of anaemia	• RET-He - RBC-He • Real-time marker for inflammation

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RET- H$_e$ for Monitoring following iv iron & erythropoetic therapy

	Day 0				Day 3				Day 9		
RBC	4.94		10A6/uL	RBC	4.91		10A6/uL	RBC	5.73	+	10A6/uL
HGB	7.1	-	g/dL	HGB	7.4	-	g/dL	HGB	9.2	-	g/dL
HCT	26.8	-	%	HCT	27.4	-	%	HCT	35.1	-	%
MCV	54.3	-	fL	MCV	55.8	-	fL	MCV	61.3	-	fL
MCH	14.4	-	pg	MCH	15.1	-	pg	MCH	16.1	-	pg
MCHC	26.5	-	g/dL	MCHC	27.0	-	g/dL	MCHC	26.2	-	g/dL
RDW-SD	43.6		fL	RDW-SD	43.4		fL	RDW-SD	44.5		fL
PLT &	574	+	10A3/uL	PLT &	514	+	10A3/uL	PLT &	369		10A3/uL
MPV	----		fL	MPV	----		fL	MPV	----		fL
P-LCR	----		%	P-LCR	----		%	P-LCR	----		%
RET#	0.0593		10A6/uL	RET#	0.1286		10A6/uL	RET#	0.1329		10A6/uL
RET%	1.20		%	RET%	2.62		%	RET%	2.32		%
IRF	11.2		%	IRF	30.0		%	IRF	22.5		%
RET-He	16.1		pg	RET-He	27.4		pg	RET-He	29.5		pg
D-He	1.8		pg	D-He	11.3		pg	D-He	13.7		pg
RPI	0.4			RPI	0.9			RPI	1.3		
% Hypo	74		%	% Hypo	77		%	% Hypo	60		%

RECENT PUBLICATION RET-H$_E$

RET-H$_e$ ideal biomarker for diagnostic of iron deficiency

Correctly identified during acute phase reaction

High clinical evidence

Retikulozyten-Hämoglobin-Äquivalent als diagnostischer Marker der aktuellen Eisendefizienz

C. Hönemann, O. Hagemann, D. Doll, M. M. L. Luedi, M. L. Ruebsam & P. Meybohm

RET-H$_E$ IN CLINICAL ROUTINE

Reference range RET-H$_e$: 27-32,3 pg

➢RET-H$_e$ < 28 pg iron deficiency
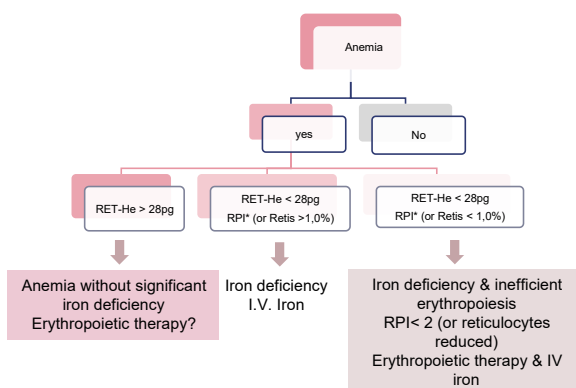➢RET-H$_e$ early indicator of treatment success within 2 to 4 days

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POSSIBLE GOOD CLINICAL MANAGEMENT OF PRE-OPERATIVE ANEMIA

STEP 1
4 weeks before the op

Laboratory diagnostics

- Hb-Wert
  → Diagnosis of anemia
  Hb< 12 g/dl Woman
  Hb< 13 g/dl Man

Iron status
→ Diagnosis of Iron deficiency
Serum-Ferritin < 30 µ/l
und/oder TSAT < 20%
oder
Serum-Ferritin 30-100 µ/l
und/oder TSAT < 20%

- KV: Hematological Practice
- Private: own ambulance

STEP 2
Identification of patients at high risk of high blood loss or transfusions
→ Optimization HB / Iron status

STEP 3
Clarification of the causes of anemia of unknown origin

STEP 4
control
- HB
- Iron status

preoperative | OPERATION | postoperative

RECOMMENDATION
Cause of the iron deficiency?

Iron supplementation for fast, effective correction of iron deficiency and / or anemia, e.g. with i.v. Iron ferinject Ⓢ

RECOMMENDATION
If necessary, additional Iron supplementation e.g. with i.v. Iron ferinject Ⓢ

Modifiziert nach Goodnough et al. 2011

TREATMENT PREOPERATIVE ANEMIA:
BEST PRACTICE PROF. HÖNEMANN

Anemia

yes | No

RET-He > 28pg | RET-He < 28pg RPI* (or Retis >1,0%) | RET-He < 28pg RPI* (or Retis < 1,0%)

Anemia without significant iron deficiency Erythropoietic therapy? | Iron deficiency I.V. Iron | Iron deficiency & inefficient erythropoiesis RPI< 2 (or reticulocytes reduced) Erythropoietic therapy & IV iron

*RPI - Reticulocyte Reproduction Index

In healthy individuals: RPI: 1-2

RPI in anemia:

> 2 → effective regeneration

> 3 → hyperregenerative erythropoiesis

$\leq$ 2 → hypoplastic / ineffective erythropoiesis

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IV. DIAGNOSTIC OF ANAEMIA AND IN CHILDREN

With Biomarkers from a bloodcount

DIAGNOSIS OF IRON DEFICIENCY

When is diagnosis of an iron deficiency particularly important?

- For patients in PBM BEFORE elective surgery, with a risk of high blood loss (saving of blood reserves, reduction of deaths and side effects)
- For patients on ICU with RET-He < 29. They have a 3-fold increased risk of transfusion and 3 days longer in hospital [1]
- For patients with chronic and acute diseases (SOC iron parameters can mask iron deficiency)
- For pregnant women (high morbidity) [2]
- For children, as they have is a risk of cerebrospinal developmental disorders not only in IDA, but also in ID

CBC requires less blood volume than measuring SOC iron parameters [3,4]

1 Fernandez et al 2010, 2 Mikhail et al 2017, Urrechaga et al. (2013), 3 Ullrich et al 2005, Mikhail et al 2017, 4 Levy et al 2018

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EXAMPLES OF RECENT PUBLICATIONS

- current publications: e.g. Hönemann et *al.* 2021, Neef et *al.* 2020 and 2021, Morton et *al.* 2022

REFERENCE INTERVALS FOR RETICULOCYTE HEMOGLOBIN CONTENT IN HEALTHY INFANTS

Löfving *et al.* 2018:

https://pubmed.ncbi.nlm.nih.gov/30140071/

Key Message:

- Ret-He reference intervals based on non-anemic and non-iron-deficient infants

- A step towards standardizing Ret-He as a pre-anemia biomarker of iron deficiency in children

- Reference intervals for newborn Ret-He:
  27.4 to 36.0 pg/L (N = 376) in the cord sample
  28.1–37.7 pg/L (N = 253) at 48–72 h
  25.6–33.4 pg/L (N = 341) at four months
  24.9–34.1 pg/L (N = 288) at 12 months

- Ret-He was significantly lower among iron-deficient infants

RET-HE DIAGNOSIS AND PREVENTION OF ID AND IDA IN INFANTS AND TODDLERS (0–3 YEARS)

Baker et al. 2010:

Clinical Report - Diagnosis and Prevention of Iron Deficiency and Iron-Deficiency Anemia in Infants and Young Children (0 –3 Years of Age)

https://pubmed.ncbi.nlm.nih.gov/20923825/

Key Message:

- Low reticulocyte hemoglobin is the strongest predictor of ID in children
- IDA and ID in infancy and childhood show long-lasting adverse effects on neurological development
- Prevalence of IDA in USA
  - for infants: 0,9 to 4,4 %
- Prevalence ID in USA
  - in infants: 4- 12%
  - in infants: 6.6 to 15.2%
- Most infants with ID do not have anemia

RET-HE AS A SCREENING TOOL OF ID AND IDA IN CHILDREN

Neef et al. 2021:

The Reticulocyte Hemoglobin Equivalent as a Screening Marker for Iron Deficiency and Iron Deficiency Anemia in Children

https://pubmed.ncbi.nlm.nih.gov/34441801/

Key Message:

- Ret-He can be used as the sole parameter for screening ID and IDA in children - without taking other iron parameters into account
- The use of Ret-He is economically highly relevant by saving one blood tube per patient and additional cost savings
- The cut-off value for Ret-He to diagnose ID was 33.5 pg

RET-HE AS A SCREENING TOOL FOR NICU PATIENTS

Screening With Reticulocyte Hemoglobin
Increased Iron Sufficiency Among NICU Patients

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7190262/

Key Message:

- Introduction of RET-H*e* as a parameter for iron supplementation in the NICU

- Doubled the number of patients discharged with normal iron status (39%)
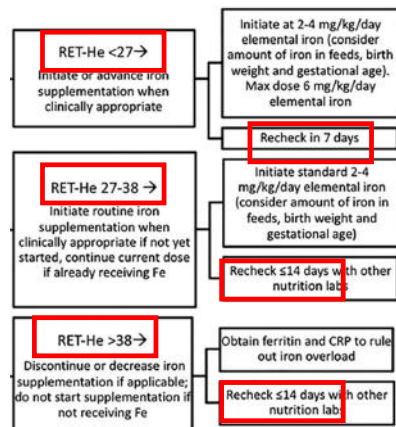
- No cases of iron overload occurred

RET-HE AS A SCREENING TOOL FOR NICU PATIENTS

Screening With Reticulocyte Hemoglobin
Increased Iron Sufficiency Among NICU Patients

http://www.nc

| RET-He <27→ Initiate or advance iron supplementation when clinically appropriate | Initiate at 2-4 mg/kg/day elemental iron (consider amount of iron in feeds, birth weight and gestational age). Max dose 6 mg/kg/day elemental iron |
	Recheck in 7 days
RET-He 27-38 → Initiate routine iron supplementation when clinically appropriate if not yet started, continue current dose if already receiving Fe	Initiate standard 2-4 mg/kg/day elemental iron (consider amount of iron in feeds, birth weight and gestational age)
	Recheck ≤14 days with other nutrition labs
RET-He >38→ Discontinue or decrease iron supplementation if applicable; do not start supplementation if not receiving Fe	Obtain ferritin and CRP to rule out iron overload
	Recheck ≤14 days with other nutrition labs

t of iron status in NICU patients.

V. DIAGNOSTIC OF INFECTIONS IN CHILDREN

With Biomarkers from a Bloodcount

AS-LYMP DISCRIMINATIVE POWER IN CHILDREN

New parameters on the hematology analyzer XN-10 (Sysmex™) allow to distinguish childhood bacterial and viral infections.
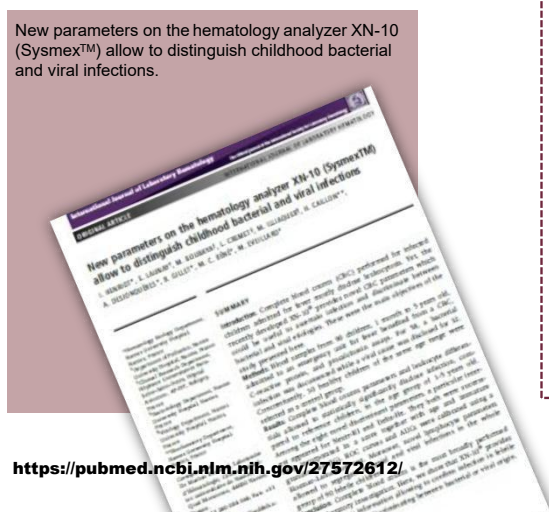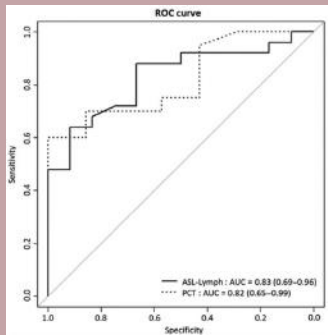
Key Message:

- 90 children (1-5 years) in the ICU, of which 58 had bacterial infections, 32 had viral infections, 30 were controls
- AS-LYMP (AUC=0.83) shows the same discriminatory power as PCT (AUC=0.82) for distinguishing between bacterial and viral infections in children

AS-LYMP DISCRIMINATIVE POWER IN CHILDREN

Henriot I *et al.* 2017:

New parameters on the hematology analyzer XN-10 (Sysmex™) allow to distinguish childhood bacterial and viral infections.

ROC curve

ASL-Lymph : AUC = 0.83 (0.69–0.96)
PCT : AUC = 0.82 (0.65–0.99)

https://pubmed.ncbi.nlm.nih.gov/27572612/

Bacterial infection:

- NEUT-RI and RE-Mono significantly increased
- Number of monocytes significantly increased
- Delta-He decreased
- CRP and PCT increased

Viral infection

- AS-Lymph and Re-Lymph increased
- AS-LMP alone: equally good discrimination
- between bacterial and viral infections
- as CRP or PCT

AS-LYMP DISCRIMINATIVE POWER IN CHILDREN

Henriot I *et al.* 2017:

New parameters on the hematology analyzer XN-10 (Sysmex™) allow to distinguish childhood bacterial and viral infections.

Condition	Points
Age in years	$18 - (3 \times Age)$
Delta-He ≤ 2	10
Neut-RI > 50	6
IG > 0.04	7

Score	Sensitivity	Specificity
>18	0.97 [0.82–1.00]	0.95 [0.82–0.99]
>22	0.83 [0.67–0.94]	1.00 [0.82–1.00]

https://pubmed.ncbi.nlm.nih.gov/27572612/

- Development of a score from age, Delta-He, NEUT-RI and IG
- Significantly improved discrimination between bacterial and viral infections

DANKE

My wife and I feel beyond honored and proud to be members of the ASPA Faculty.

Thank you so much to all participants, to the faculty and to

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