儿童溶血性贫血的诊断思路

Diagnostic Approaches to Hemolytic Anemia

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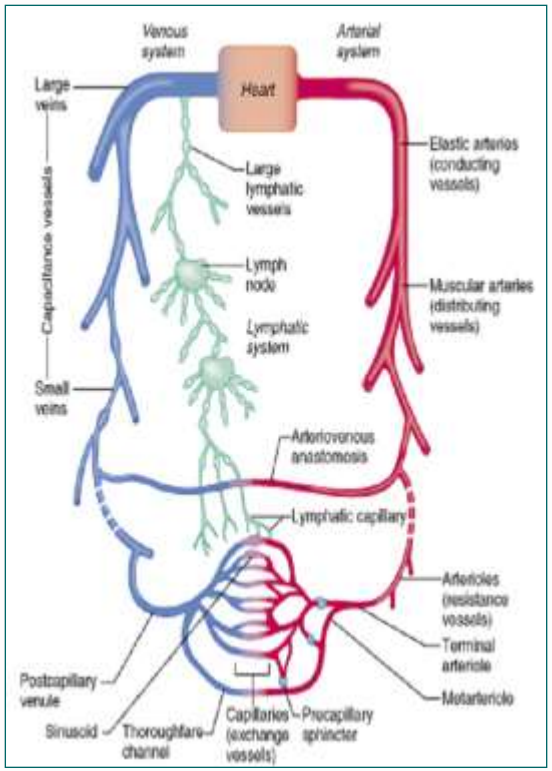
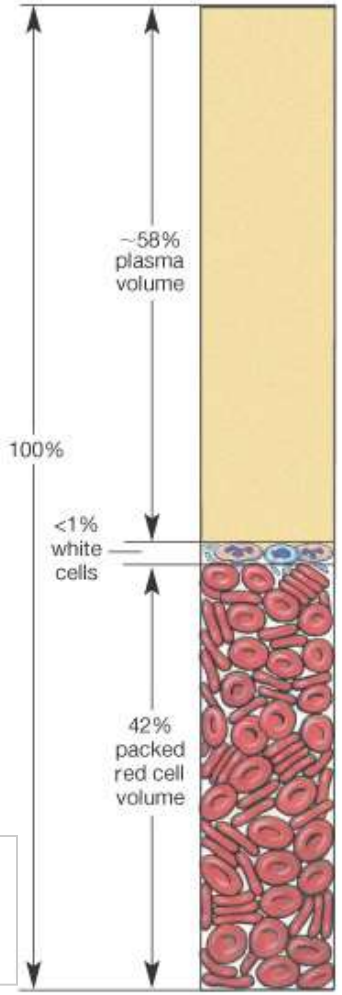
主要内容

- 血液学基础（Essentials of Hematology）
- 溶血性贫血的定义和分类（Definition and Classification）
- 溶血性贫血的诊断步骤（Diagnostic Steps）
- 溶血性贫血的诊断流程（Diagnostic Approaches to HA）
- 小结（Brief Summary）

血液学基础（Essentials of Hematology）

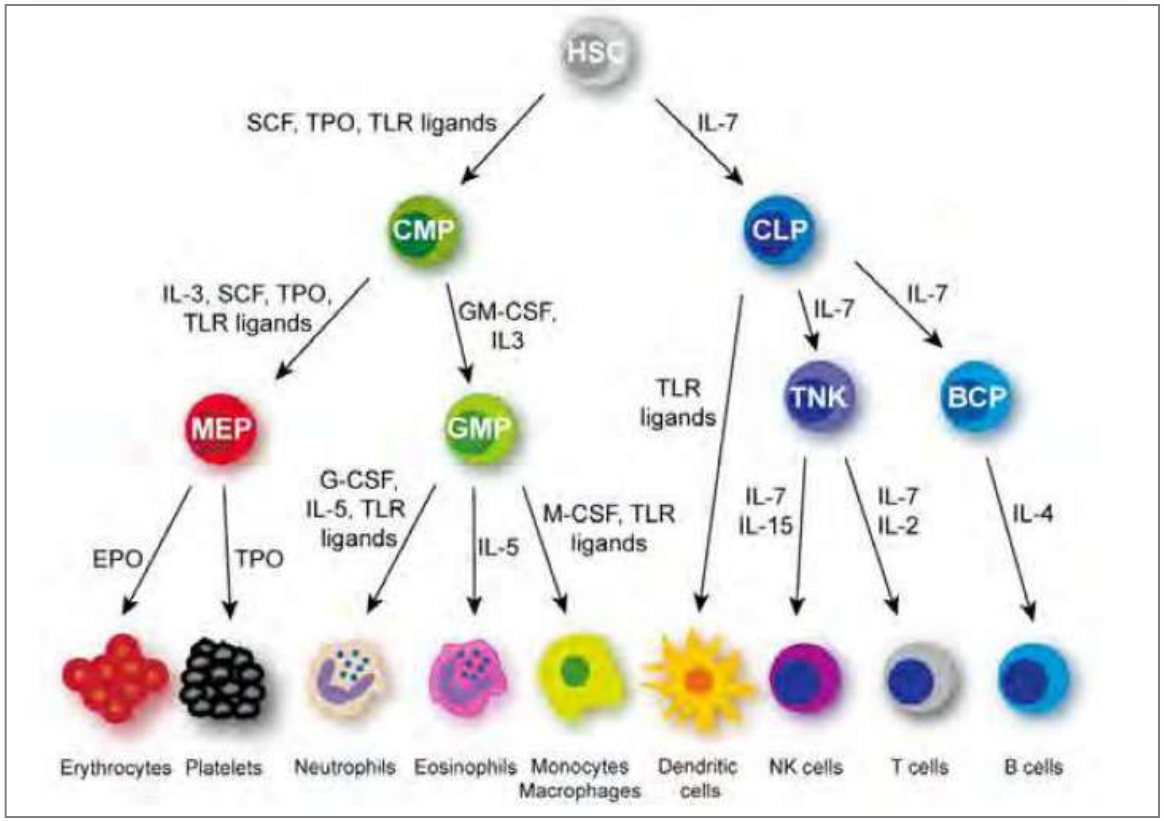
- 氧气运输（oxygen transportation）为血液最重要生理功能
- 是由红细胞（Hb）介导完成的





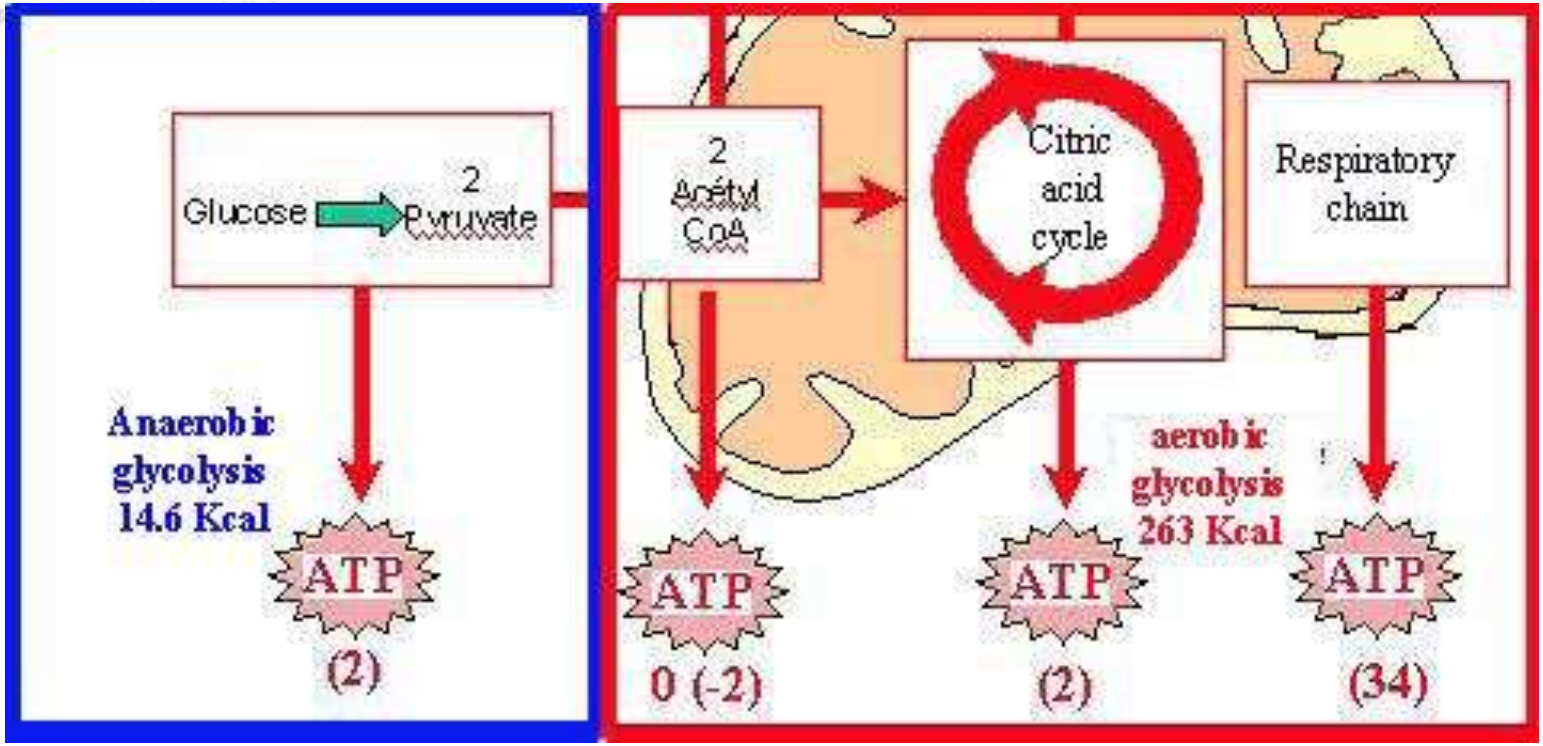
- Hct：0.42-0.52(男性)；0.37-0.47 (女性)

RBC production and destruction are kept in dynamic balance (P=D)



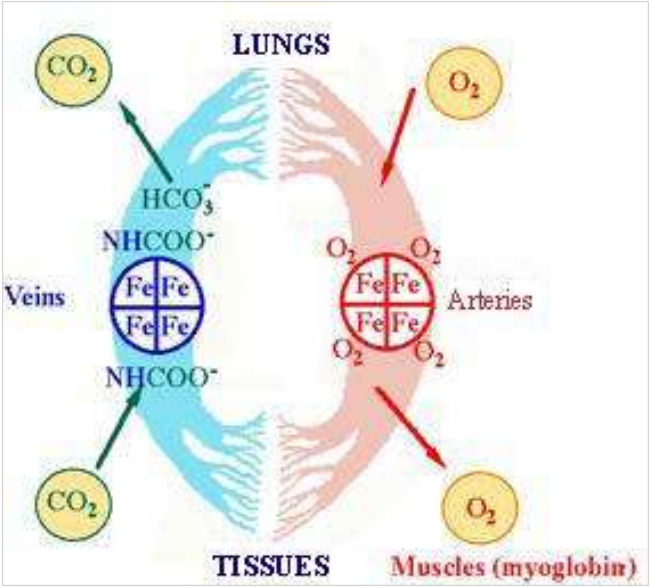
- ✓ 70kg成人血容量5L
- ✓ 红细胞5百万/μL，总数为25万亿，占人体细胞总数50%
- ✓ 外周血成熟红细胞平均寿命120天
- ✓ 每天1/120红细胞被破坏2千亿（200 billion），每秒超过2百万。
- ✓ 同时生成相同数量红细胞

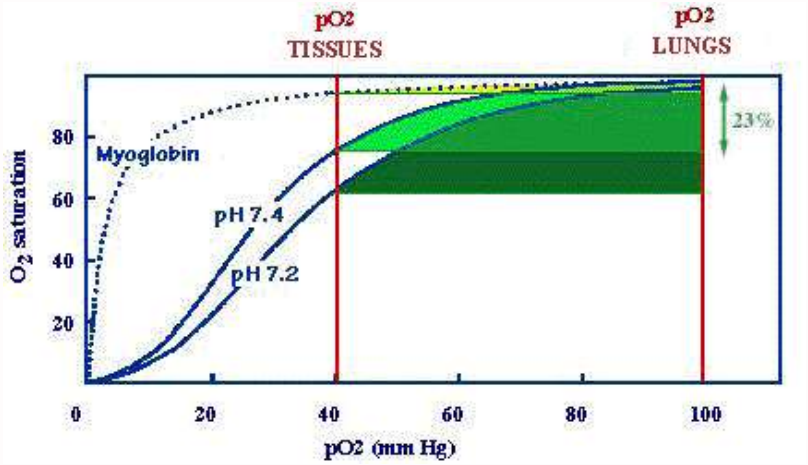
HSC: hematopoietic stem cell; CLP: common lymphoid progenitor; CMP: common myeloid progenitor; MEP: megakaryocyte-erythrocyte progenitor; GMP: granulocyte-macrophage progenitor; TNK: T cell and NK cell progenitor; BCP: B cell progenitor



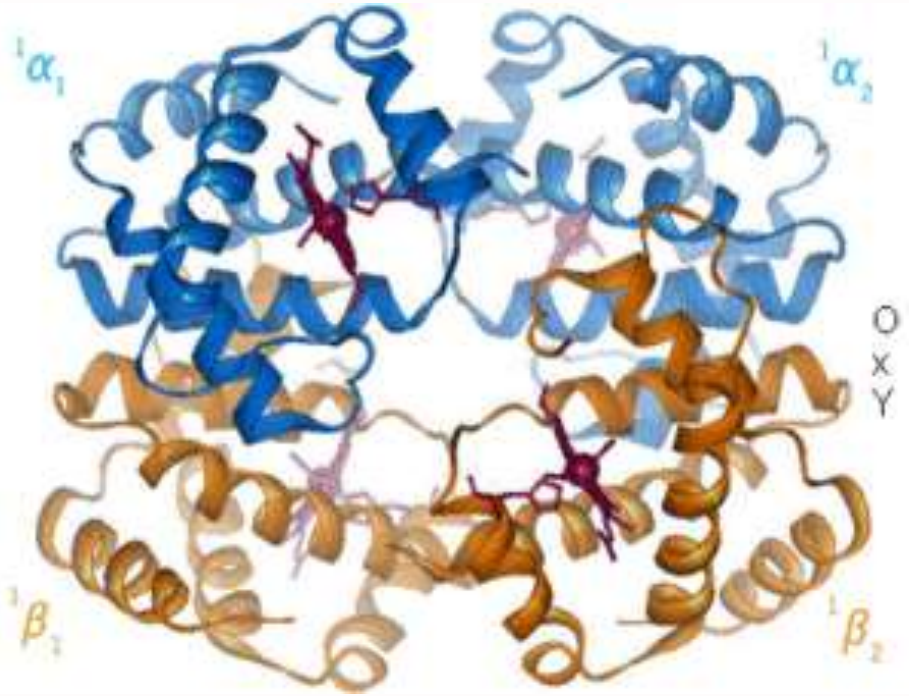
- 人类为绝对需氧动物（strict aerobic animal），需要氧气参与能量代谢维持生命。线粒体三羧酸循环为能量产生的主要途径
- 1分子葡萄糖经无氧酵解(anaerobic glycolysis) ATP净产量=2分子；而有氧代谢（三羧酸循环）ATP净产量=36分子

Hb is just the right vehicle for oxygen carrying

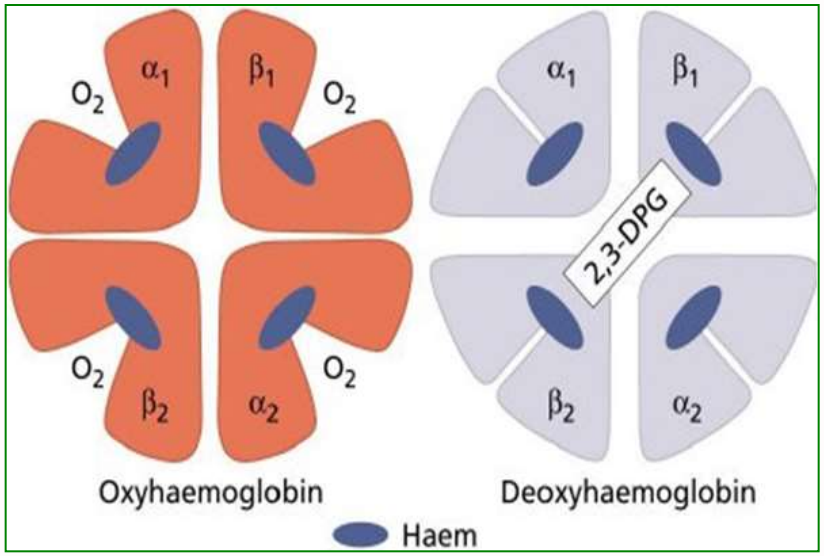


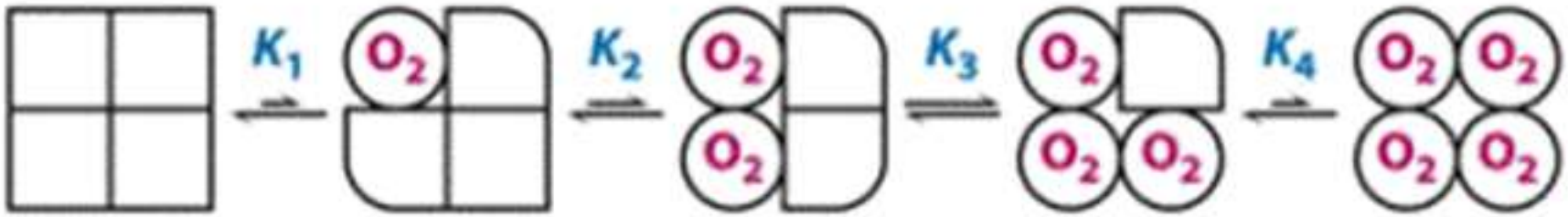


- 分子结构特点（变构酶, allosteric enzyme）
- S形氧离曲线（sigmoidal oxygen dissociation curve），有利于在高氧分压的肺脏与O_2分子结合，同时在低氧分压的外周组织释放出O_2分子-协同效应（cooperativity）

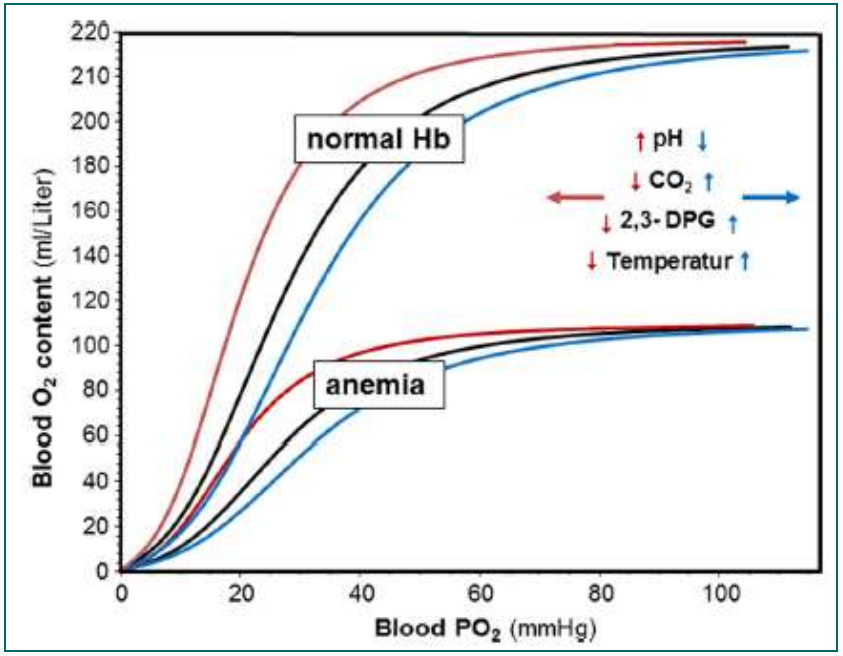


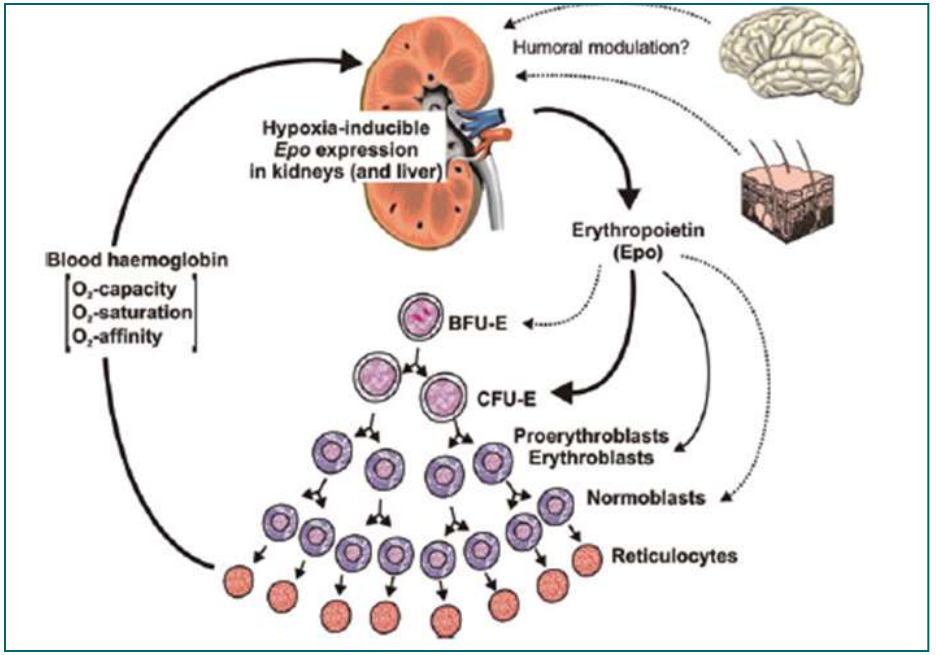
Hb协同效应（Hb cooperativity）





- Hemoglobin exists in two distinct states. **T-state** (tense or deoxyhemoglobin state) and **R-state** (relax or oxyhemoglobin state)





Environmental oxygen tension

Alveolar oxygen pressure

Cardiac output and tissue blood flow

Blood oxygen content

Oxygen extraction by tissue cells

Tissue oxygenation and supply

Tissue oxygen supply depends not only on Hb content, but also on Hb structure and oxygen affinity

Hb is just the right vehicle for oxygen carrying

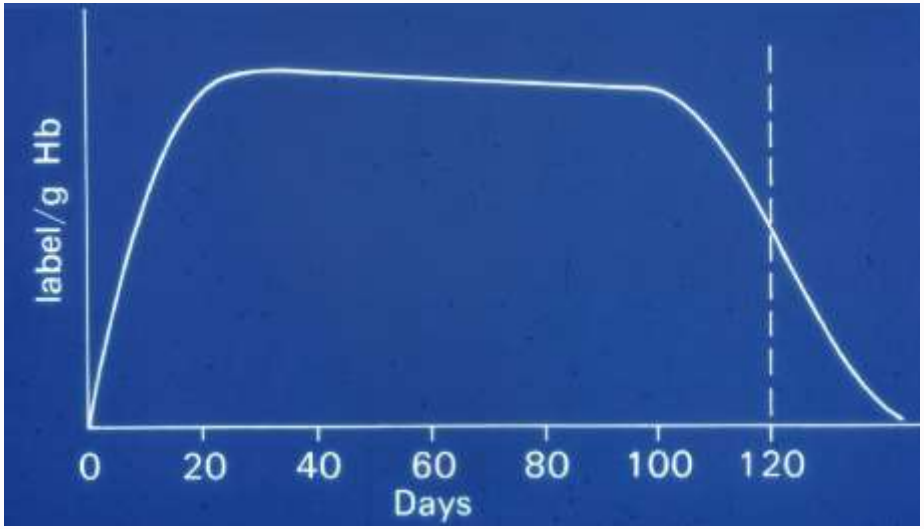
- 由于氧的理化性质，必须要有一种合适的“运载工具”，担负氧气运输这一重要生理功能
  - ✓ 氧气水溶解度低（low oxygen solubility）：动脉血最大氧分压100 mmHg情况下，100ml 水（血浆）中O_2溶解量（氧含量）仅0.3ml；而1g Hb携氧量1.34ml，按Hb浓度15g/dl计算100ml血液携氧量为20ml（相差60倍）
  - ✓ 氧气在水中的弥散度差(poor diffusibility)

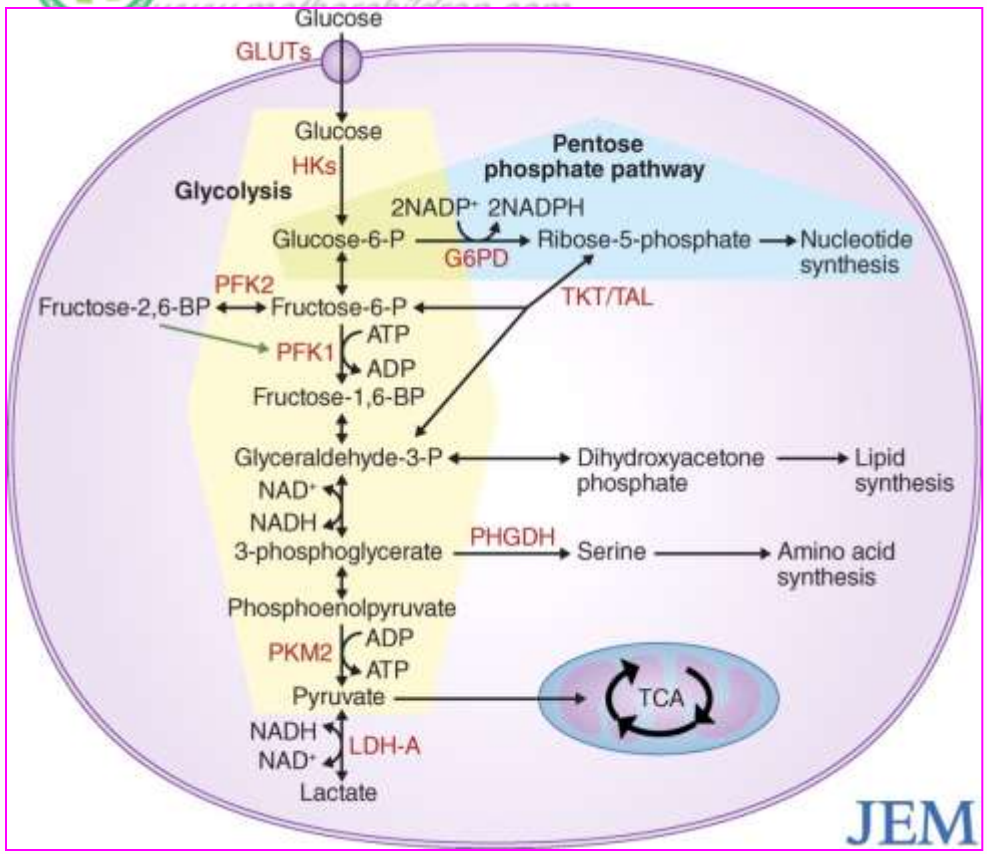
Henry’s law : 氧分压100mmHg情况下，100ml血液氧含量=

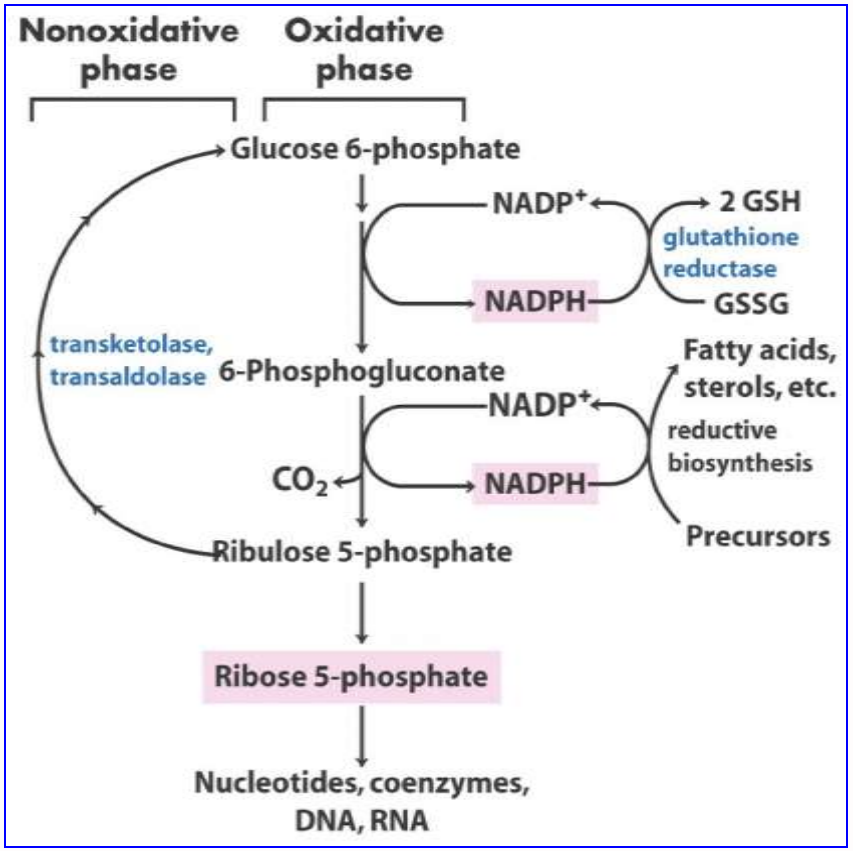
$$[1.34 \times Hb(g/dL) \times (SaO_2/100)] + 0.003 \times PO_2 = 20.8ml$$

成熟RBC的结构和代谢特点

- 无细胞核：无分裂能力，寿命注定仅100-120天
- 无线粒体：不能进行Kreb Cycle，能量产生依赖于无氧酵解
- 无核糖体：无蛋白合成能力
- 盘状双凹形态（biconcave disc），表面积与体积之比最大，可塑性强（deformability），而且有利于氧气的弥散

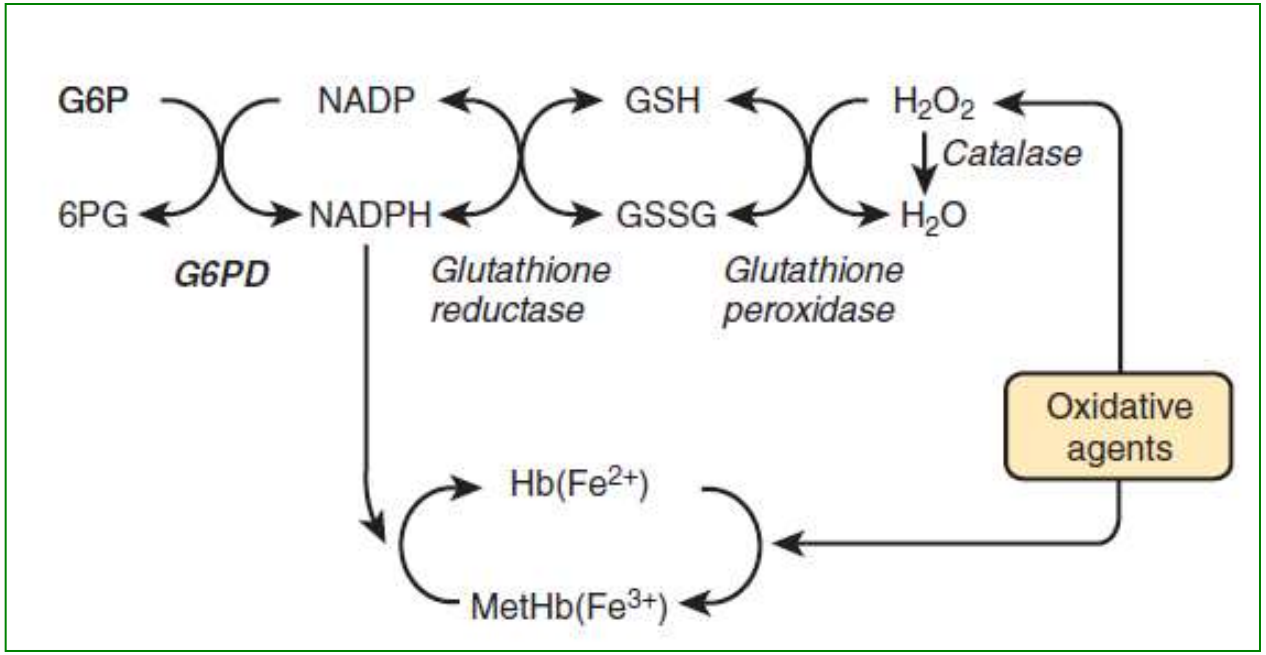






- G6PD：磷酸戊糖途径(pentose phosphate pathway, PPP)或单磷酸己糖旁路(hexose monophosphate shunt，HMP)第一个酶及限速酶，与底物NADP亲和力和特异性很高
- G6PD的关键代谢作用： 保障细胞辅酶Ⅱ (NADPH)的产生，催化“还原型谷胱甘肽”(GSH)的产生

辅酶Ⅱ（NADPH）为细胞抗氧化应激损伤的关键分子

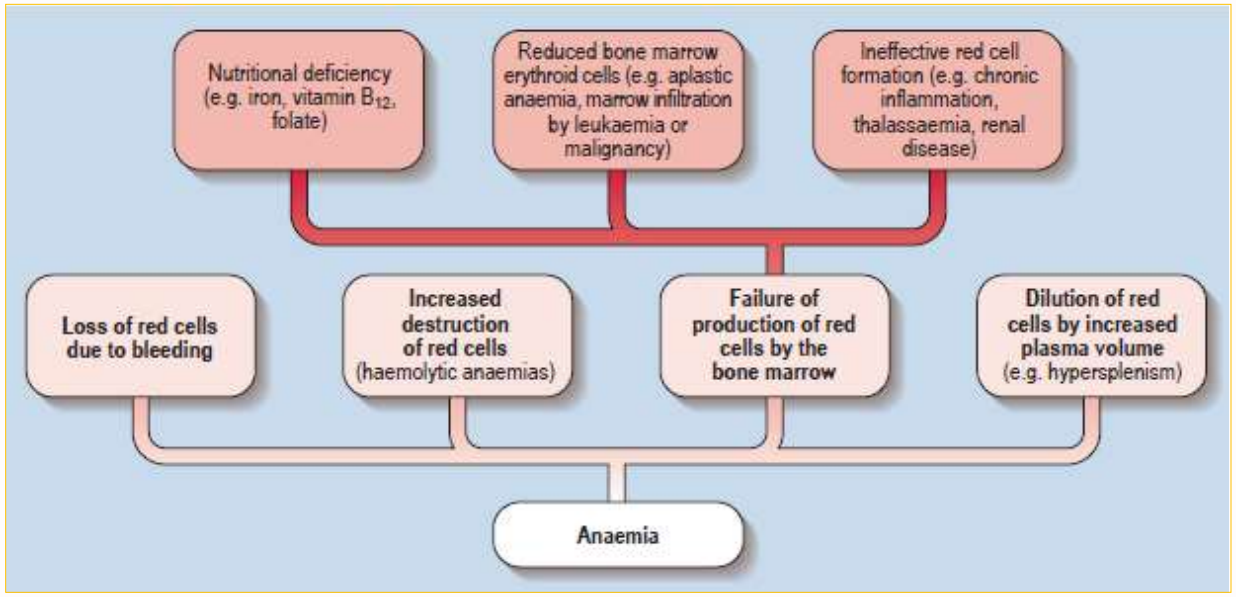


- 正常情况下红细胞中GSSG/GSH=100:1
- NAPDH为谷胱甘肽还原酶活性所必须
- GSH保护Hb巯基被氧化以及细胞膜的脂质过氧化
- GSH减少必将导致氧化应激损伤

- 超氧化物歧化酶(superoxide dismutase, SOD) 将过氧化物(peroxide)转化为过氧化氢(H_2O_2)； 过氧化氢酶(catalase)和谷胱甘肽过氧化物酶(glutathione peroxidase)将过氧化氢(H_2O_2)催化为水(H_2O)
- NADPH为谷胱甘肽还原酶的底物，维持catalase活性构象

溶血性贫血的定义

- 溶血性贫血（hemolytic anemia）：RBC的内在缺陷或外在不良因素导致外周血RBC破坏加速、寿命缩短的一类贫血的总称
- Hemolytic anemia is caused by premature destruction of RBCs in the peripheral circulation due to RBC intrinsic defects or RBC extrinsic noxious factors, resulting in a reduction of the average life-span of red blood cells.

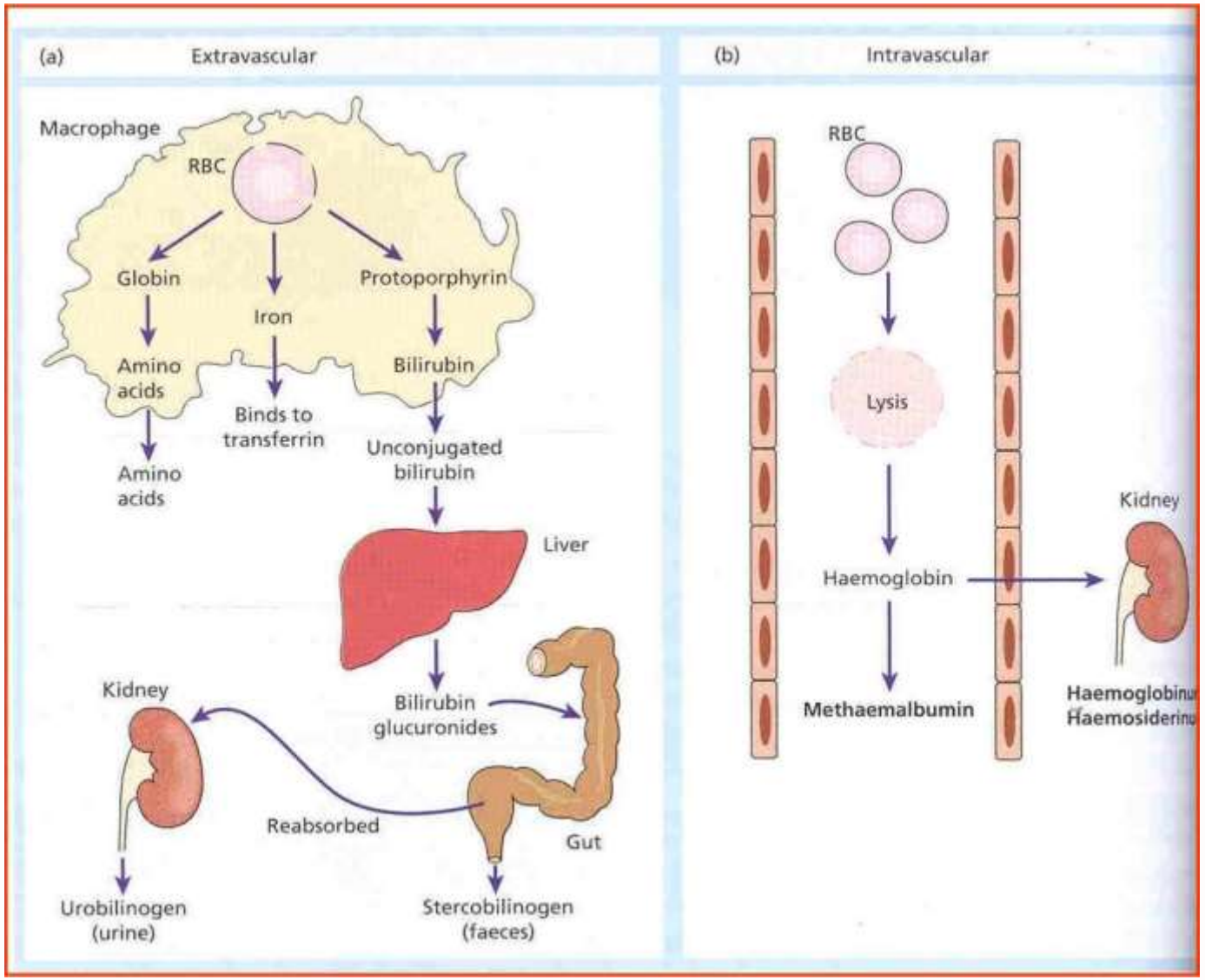


- 临床常见贫血类型，病因和发病机制复杂，但不是一种独立的疾病
- 溶血（红细胞破坏）不一定导致溶血性贫血，D＞P为基本条件
- 实验室检查在溶血性贫血诊断和鉴别方面具有极为重要的价值

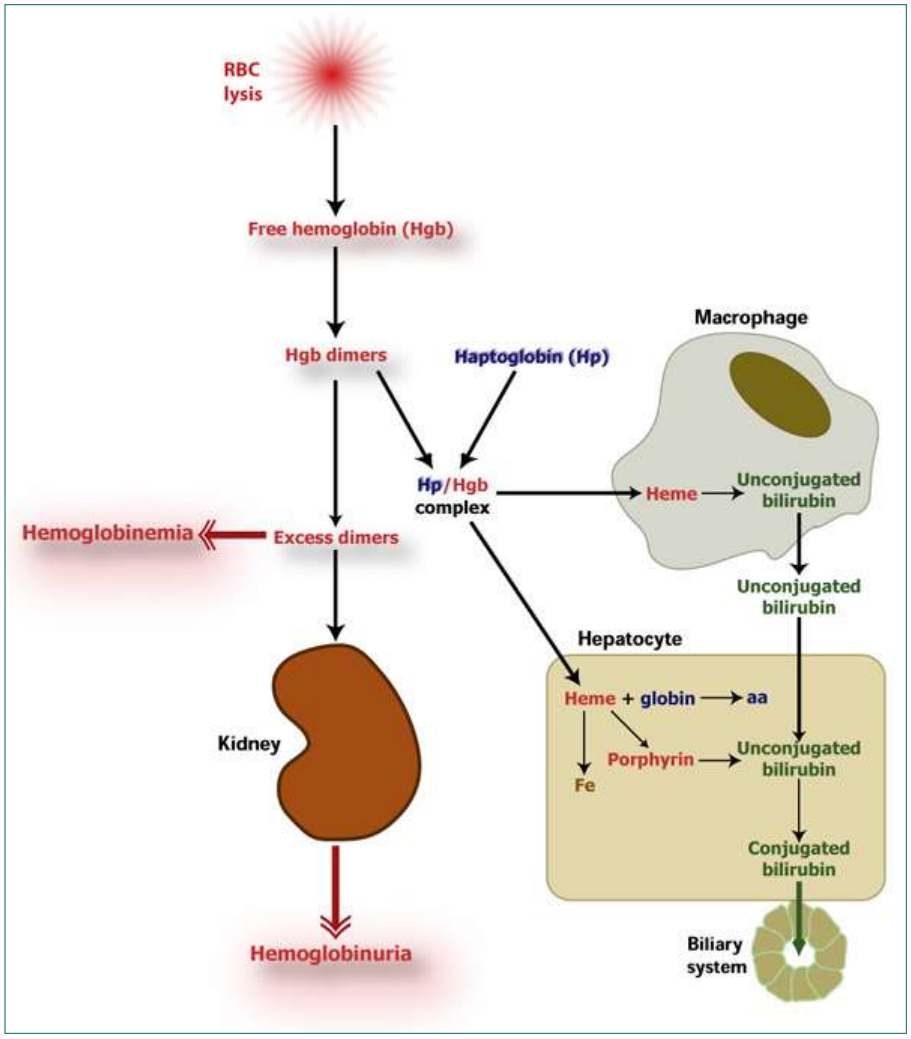
Table 16-3: Causes of Anemia

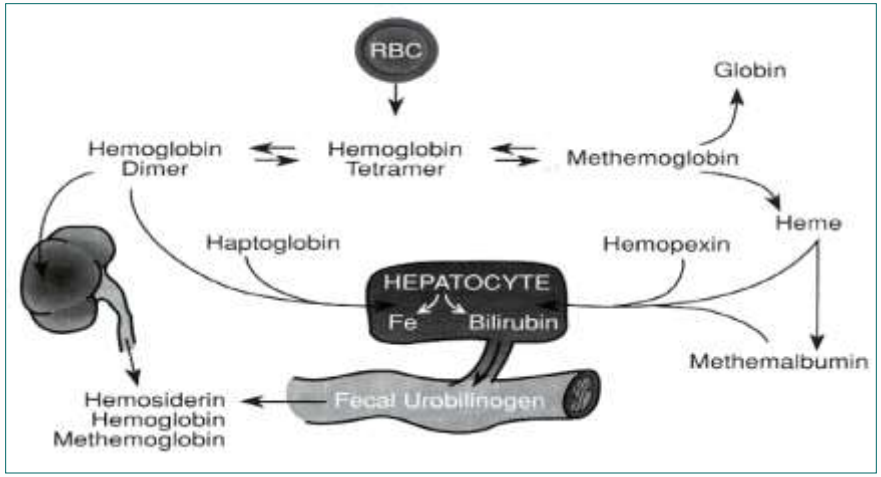
Accelerated red blood cell loss
Blood loss: Cells are normal in size and hemoglobin content but low in number
Hemolytic anemias: Cells rupture at an abnormally high rate
Hereditary
Membrane defects (example: hereditary spherocytosis)
Enzyme defects
Abnormal hemoglobin (example: sickle cell anemia)
Acquired
Parasitic infections (example: malaria)
Drugs
Autoimmune reactions
Decreased red blood cell production
Defective red blood cell or hemoglobin synthesis in the bone marrow
Aplastic anemia: Can be caused by certain drugs or radiation
Inadequate dietary intake of essential nutrients
Iron deficiency (iron is required for heme production)
Folic acid deficiency (folic acid is required for DNA synthesis)
Vitamin B_{12} deficiency. May be due to lack of intrinsic factor for B_{12} absorption (vitamin B_{12} is required for DNA synthesis)
Inadequate production of erythropoietin

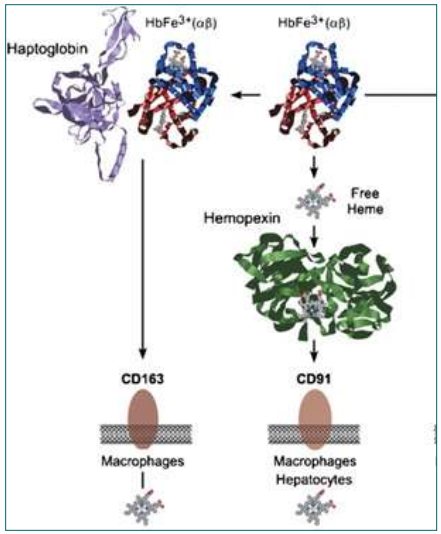
溶血性贫血的分类(classification)

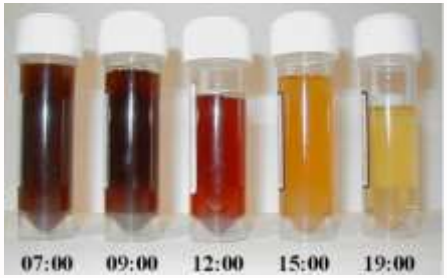


血管内溶血（Intravascular Hemolysis）





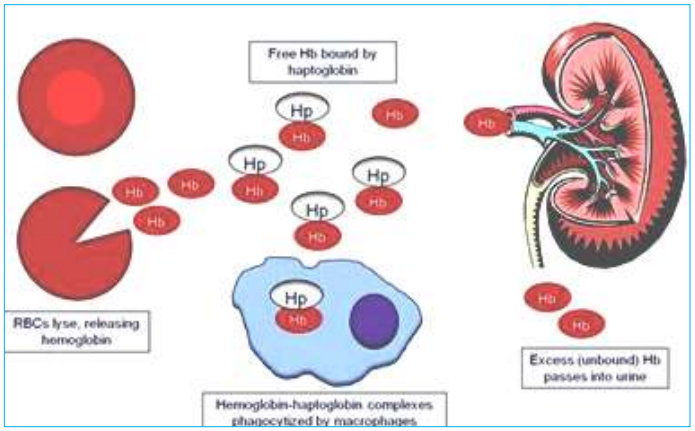


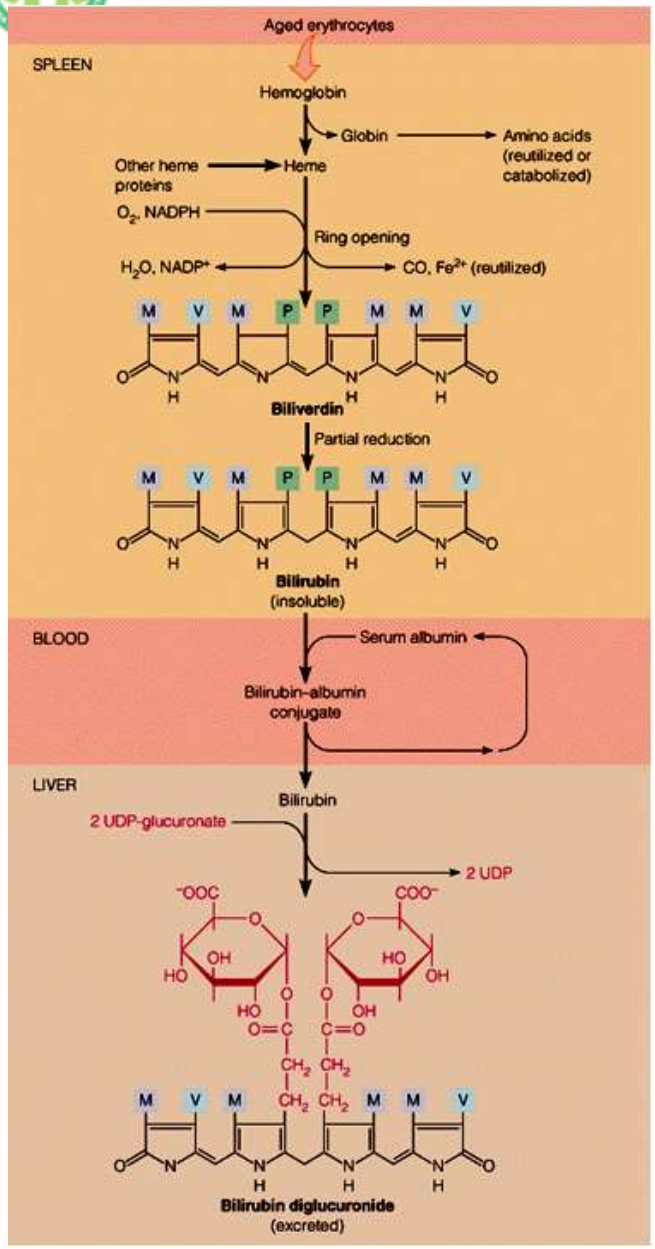


Hemoglobinuria in paroxysmal cold hemoglobinuria (PCH) and cold agglutinin syndrome (CAS)

Intravascular hemolysis: characteristics

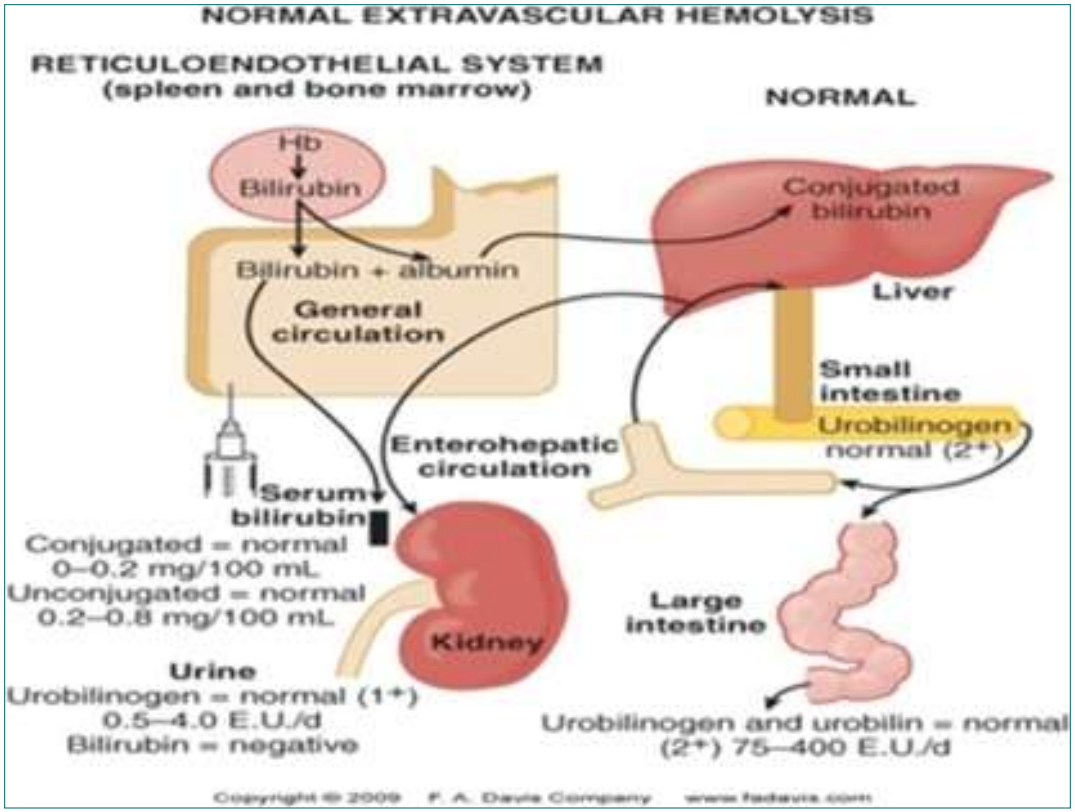
- Free hemoglobulinemia
- Reduced or absent haptoglobin (Hp)
- Reduced or absent hemopexin
- Hemoglobinuria (wine-like urine)
- hemosiderinuria





血管外溶血

- 红细胞破坏主要发生于MMS
- 无血红蛋白血症和血红蛋白尿症
- MMS增生导致脾大，黄疸较明显



血管外溶血与血管内溶血的比较

	Extravascular hemolysis	Intravascular hemolysis
Frequency	Most common	Less common
Etiology	Reduced RBC deformability resulting in splenic trapping and destruction	Direct damage by mechanical injury or bacterial toxin. Complement fixation
Jaundice	More severe	Generally mild
Splenomegaly	More prominent	Less remarkable
Serum bilirubin	↑ Unconjugated	↑ Unconjugated
Seum haptoglobin	↓ or absent	absent
Hemoglobinemia	Normal or ↑	↑↑
Serum LDH	Variable ↑	Variable to ↑↑
Urine bilirubin	0	0
Urine hemosiderin	0	+
Urine hemoglobin	0	+ (in severe cases)

- 根据溶血的原因
  - ✓ 红细胞内在缺陷所致的溶血 (intrinsic defects)
  - ✓ 红细胞外在因素所致的溶血 (extrinsic noxious factors)
- 根据溶血的发生机制
  - ✓ 遗传性溶血(inherited hemolysis)
  - ✓ 获得性溶血 (acquired hemolysis)

- 红细胞内在缺陷所致一般为遗传性和先天性
- 红细胞外在不良因素所致多为后天性或获得性

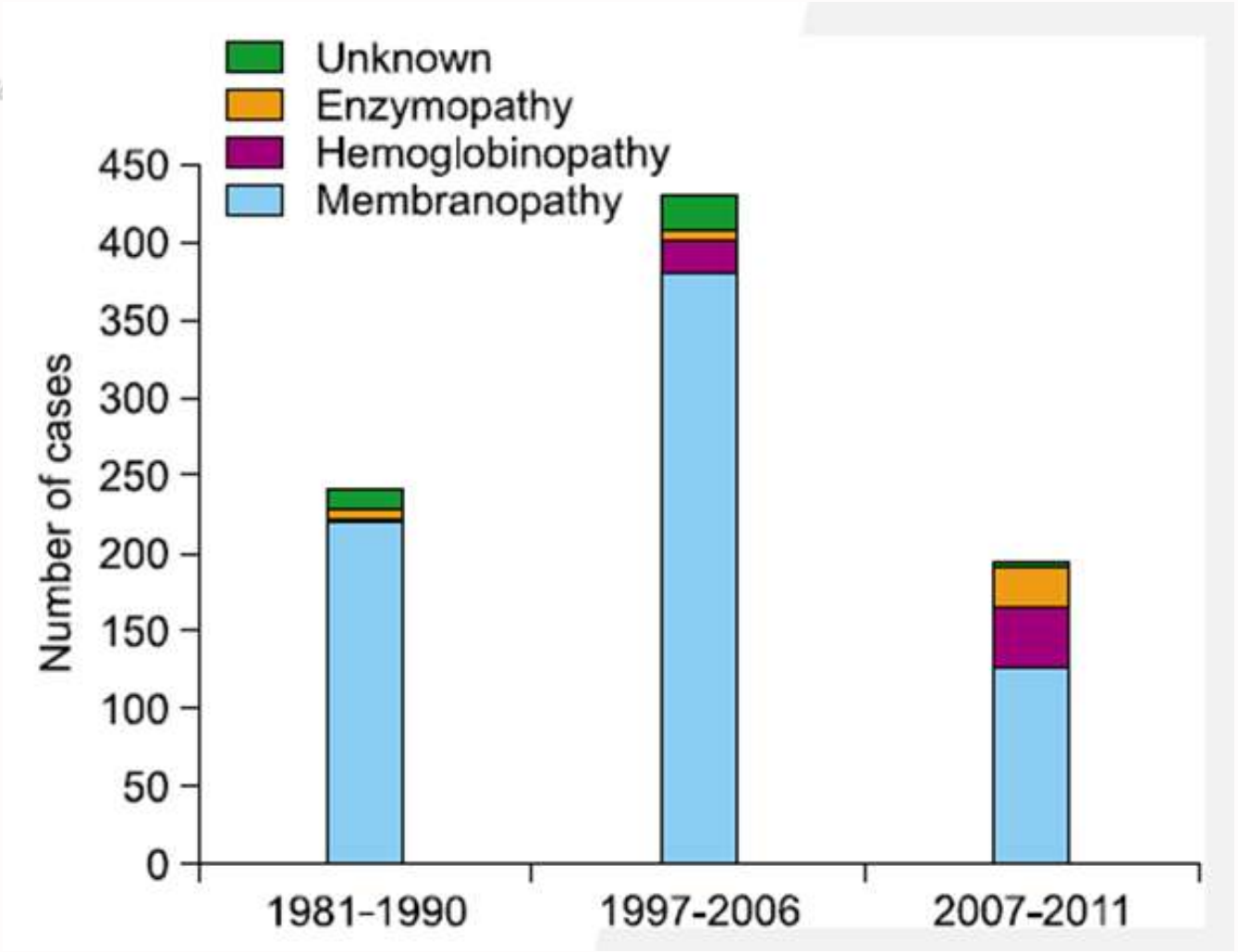
溶血性贫血的病因学分类

一、先天性或遗传性溶血性贫血

- RBC膜缺陷：遗传性球形细胞增多症（HS）；椭圆形、口形、棘形红细胞增多症
- RBC酶缺陷：G6PD缺乏症；PK缺乏症
- 核苷酸代谢缺陷：嘧啶5’-核苷酸酶（极为罕见）
- Hb珠蛋白缺陷
  - 结构异常(质量异常)：（窄义性）血红蛋白病 (hemoglobinopathy)，如SCA
  - 合成障碍(数量异常)：地中海贫血

二、后天性或获得性溶血性贫血

- 免疫性溶血性贫血：同种免疫性（新生儿溶血病）或自身免疫溶血性贫血
- 药物相关性溶血性贫血：免疫性或非免疫性
- 毒物或代谢异常所致：慢性肾衰贫血
- RBC内寄生虫：疟疾，黑热病
- RBC机械性损伤：DIC；心脏换瓣术后、溶血尿毒综合征和其他微血管病性溶血性贫血
- 脾功能亢进：Sequestrational hemolysis
- PNH



韩国遗传性溶血性贫血流调结果显示，红细胞酶病和血红蛋白病发病率构比增高，与诊断水平提高相关

Jung HL. A new paradigm in the diagnosis of hereditary hemolytic anemia. Blood Res. 2013; 48(4): 237-239.

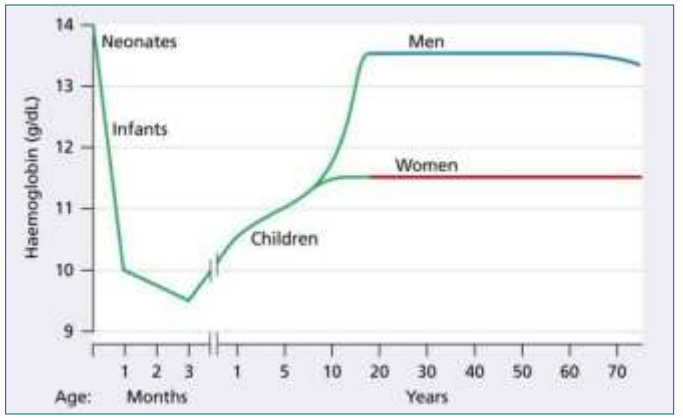
溶贫的诊断步骤（Diagnostic Steps）

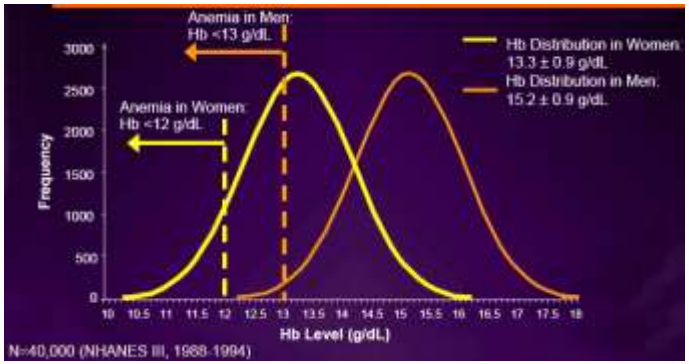
- 有无贫血（Is there anemia indeed）？
- 程度如何（How about the degree）？
- 是否为溶血性贫血（whether the anemia hemolytic）？
- 溶血性贫血类型如何（What type of hemolytic anemia）？
- 溶血性贫血病因（What is the underlying cause）？

Stepwise Diagnostic Approaches to Hemolytic Anemias

有无贫血及其程度

- WHO (1972年)(海平面)
  - 6月-6 岁 <110g/L
  - 6岁-14岁<120g/L
  - 新生儿<145g/L（全国新生儿会议1982年）
  - 成年男性<130g/L
  - 成年女性<120g/L
  - 妊娠期贫血<110g/L
- 正常人群和贫血人群Hb分布曲线间存在重叠





Stepwise Diagnostic Approaches to Hemolytic Anemias

首先明确有无溶血

红细胞破坏增多的证据

- Hb分解代谢↑

- ✓ 贫血(RBC, Hb和Hct↓)
- ✓ 外周血红细胞大小不等、甚至出现红细胞碎片
- ✓ 黄疸，血清总胆↑, 间胆为主
- ✓ 血清LDH↑
- ✓ 血红蛋白血症和血红蛋白尿症
- ✓ 结合珠蛋白和或血结素↓
- ✓ 红细胞寿命缩短(^{51}Cr labeling)

红系造血代偿增生的证据

- 红细胞代偿性生成↑

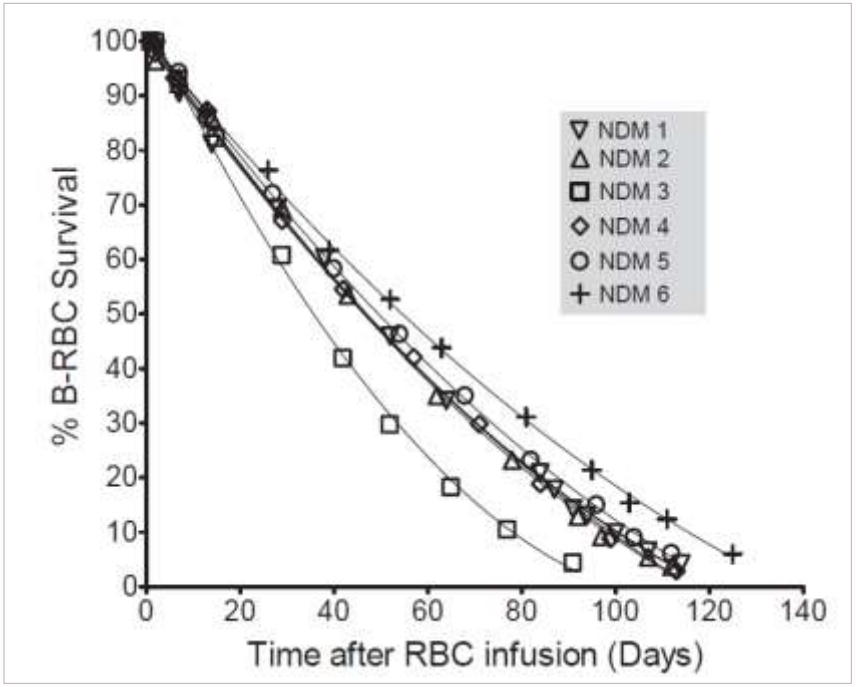
- ✓ 骨髓红系增生明显活跃，粒红比例降低甚至倒置
- ✓ 外周血出现有核RBC，网织红比例增高、绝对计数升高
- ✓ 髓外造血增加：肝、脾、淋巴结肿大
- ✓ 长期慢性溶血所致骨骼畸形、胆色素性胆结石

红细胞破坏增多的证据：红细胞寿命测定

- ✓ Shortened red cell lifespan：诊断溶血性贫血最直接的证据
- ✓ ^{51}Cr, ^{99m}Tc or ^{131}I radiolabeling：Clinically impractical (radioactivity)
- ✓ Non-radiolabeling methods: biotin-labeling, glycin-incoporation and CO production, ect.

Major Applications of Red cell labeling

- ✓ Measurement of RBC mass/volume
- ✓ Measurement of RBC survival time
- ✓ Documentation of RBC destruction
- ✓ Blood pool imaging (GI bleeding)
- ✓ Selective spleen imaging with damaged red cells

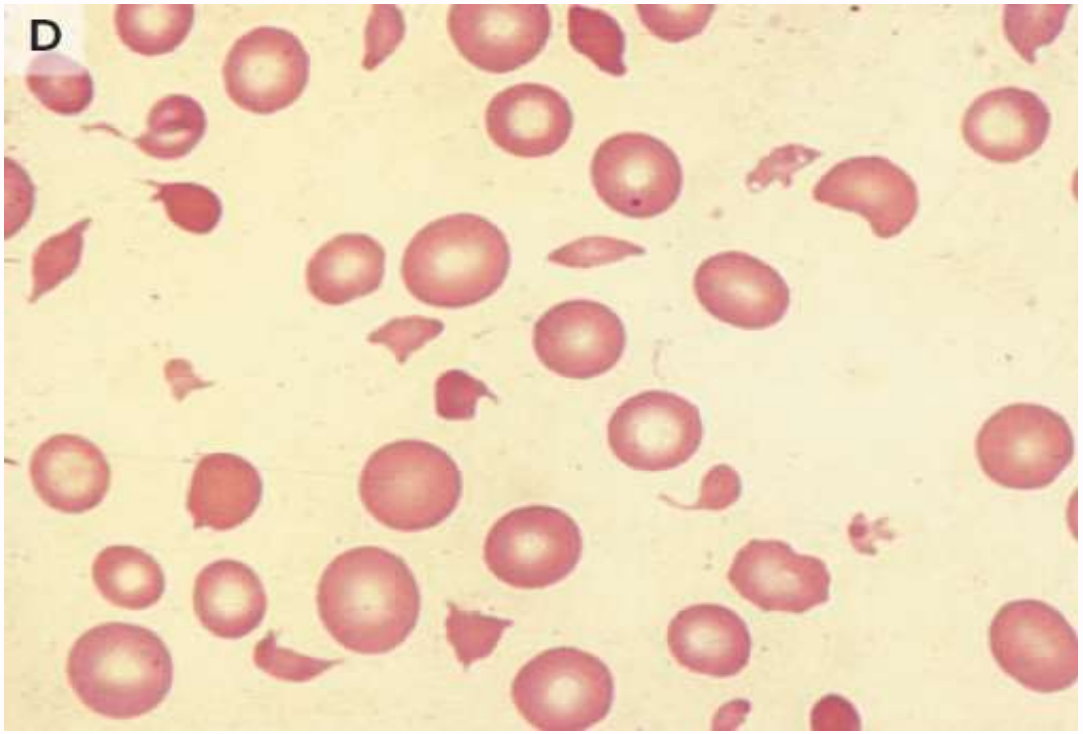


Franco RS. Measurement of Red Cell Lifespan and Aging. Transfus Med Hemother 2012;39:302-307.

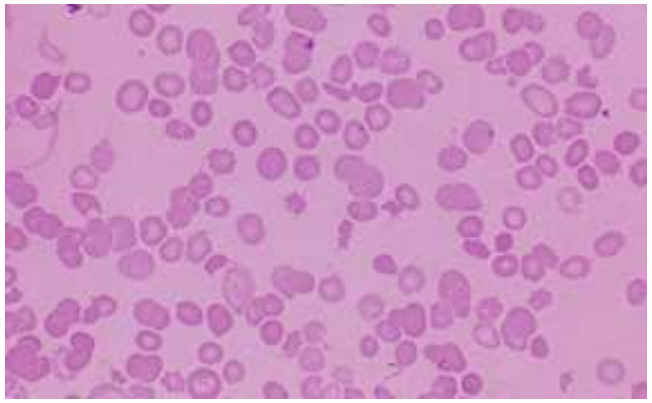
红细胞破坏增多的证据：贫血和异形红细胞

✓ 贫血：必要和首要条件
✓ 红细胞碎片或裂细胞

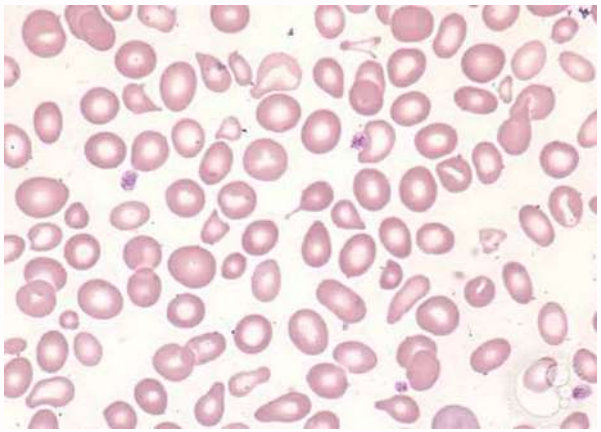
✓ 红细胞大小不等
✓ 异形红细胞增多



Numerous schistocytes （red cell fragments）
documented in microangiopathic hemolytic anemia



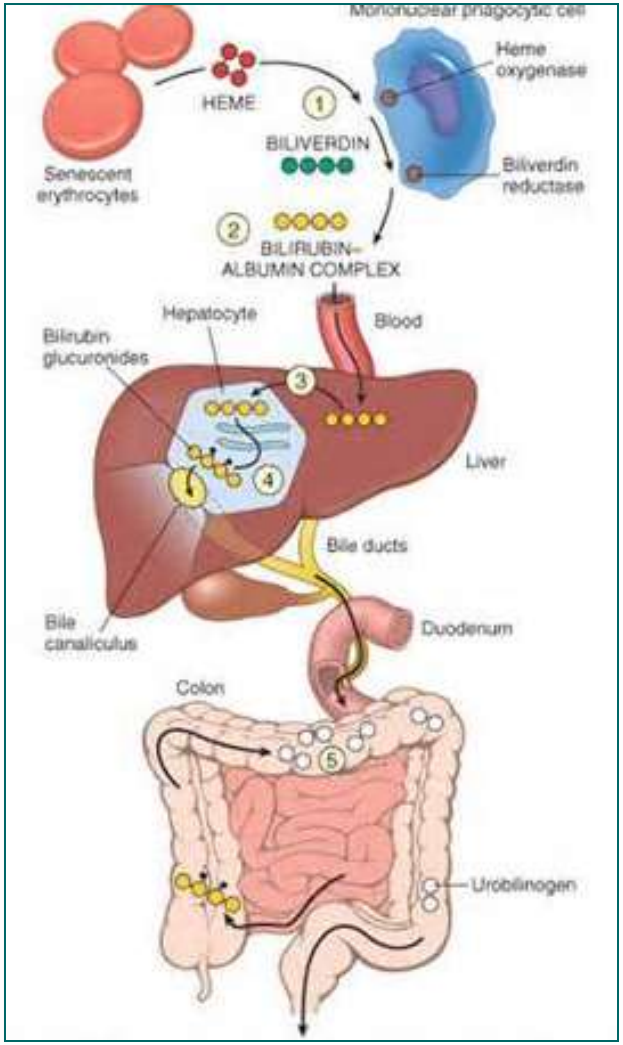
红细胞大小不等（anisocytosis）



异形红细胞增多
（poikilocytosis）

红细胞破坏增多的证据：血液生化指标

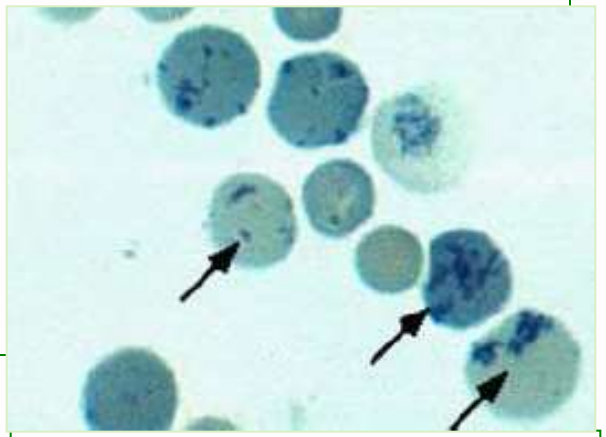
- 高胆红素血症（hyperbilirubinemia），间接胆红素升高为主
  - ✓ 敏感但非特异性指标
  - ✓ 血清间接胆红素水平与Hct密切相关。Hct45%时正常上限约1mg/dL；Hct22.5%时正常上限约0.5mg/dL
- 血清乳酸脱氢酶（LDH）升高：敏感但非特异性指标
- 血清结合珠蛋白降低和含铁血黄素尿：特异但不敏感



- ↑LDH and ↓haptoglobin： 90% specific for the diagnosis of hemolysis
- Normal LDH and normal haptoglobin (>25mg/dL): 92% sensitive for excluding haemolysis

红细胞代偿增生的证据

- ✓ 网织红细胞计数（Reticulocyte count）：反映骨髓红系造血增生能力、鉴别增生性和低增生性贫血的快速和简易指标
- ✓ 升高见于：溶血性贫血和失血性贫血
- ✓ 降低见于：骨髓衰竭性疾病，IDA，ACD等
- ✓ **Normal reticulocyte percentage：0.5%~1.5%**
- ✓ 网织红绝对计数=网织红（%）×红细胞数
  成年男性正常值：**(24-84)×10^9/L**



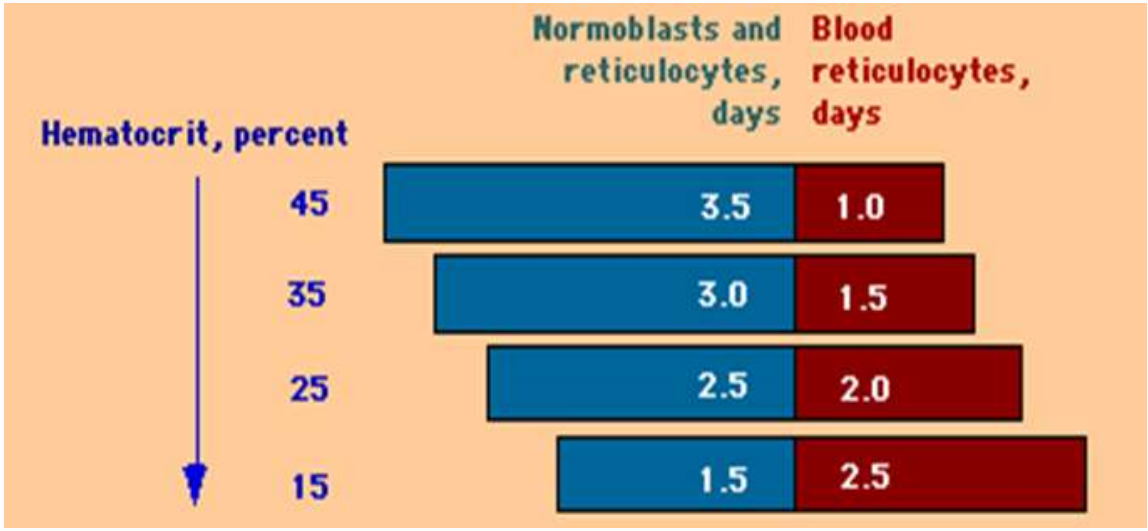
Relative number (reticulocytes ‰, %) ->	life span of red blood cells
Cell concentration (reticulocytes /µL) ->	erythropoietic productivity

- ✓ Immature RBCs containing remnant RNA and organelles
- ✓ Ribosomal and residual RNA coprecipitate with remaining mitochondria and ferritin to form dark-blue clusters and filaments (reticulum) when stained with supra-vital dyes

- Ret Index (RI) = Ret count (%) × (Hct测定值 / Hct正常值)
- Ret Production Index (RPI) = RI / Maturation Factor（纠正网织红细胞提前释放入血寿命延长的影响）

RPI有助于鉴别增生性、增低生性和增生正常性贫血

成熟因子或纠正指数（correction factors，CF）	
hematocrit（%）	maturation factor (网织红细胞外周血生存天数)
40-45	1.0
35-39	1.5
25-34	2.0
15-24	2.5



- 贫血情况下，如 RPI < 2.0，表明骨髓红系造血代偿不足（hypoproliferative），见于骨髓增生低下或造血原料不足等
- 贫血情况下，如RPI > 3.0，表明骨髓红系造血代偿充分（hyperproliferative），多见于失血或溶血

- RPI < 2 hypoproliferative (inadequate response)

- Iron def. anemia
- B12/folate def.
- Chronic disease
- Sideroblastic anemia
- Aplastic anemia
- Myeloproliferative

- RPI >= 2 : hyperproliferative (adequate response)

- Hemolytic disease
- Hemoglobinopathy (including thalassemia)
- Treated B12/folate def.

	Patient A	Patient B	Patients C	Patient D
Haemoglobin	130g/L	50g/L	75 g/L	72 g/L
Erythrocyte Count	4.4 million/mm^3	1.2 million/mm^3	2.5 million/mm^3	3.5 million/mm^3
Hematocrit	40.48%	16.2%	23.25%	25.2%
MCH	29.55 pg	41.67 pg	30 pg	20.57 pg
MCV	92 fl	135 fl	93 fl	72 fl
MCHC	32 g/dL	31 g/dL	32 g/dL	29 g/dL
Reticulocyte Count	1.3%	3%	15%	2%
Absolute Reticulocyte count	57200 /mm^3	36000/mm^3	375000/mm^3	70000/mm^3
Corrected Reticulocyte Count	1.17	1.08	7.75	1.12
Reticulocyte Production Index	0.78	0.43	3.1	0.56

- Reticulocyte Production Index (RPI) helps to determine if bone marrow is properly responding to the body's need for red blood cells
- RPI <2 indicates inadequate BM erythropoietic response

- **未成熟网织红细胞比例（Immature Reticulocyte Fraction, IRF）：反映骨髓红系增生能力的早期指标（early marker for evaluating erythropoietic regeneration），比网织红细胞升高早**

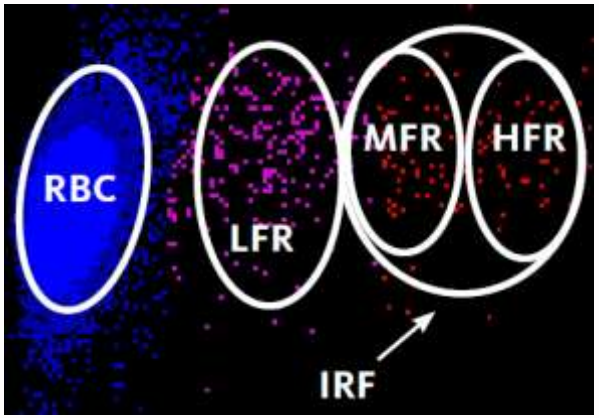
Maturation stages of reticulocytes as determined by flow cytometry

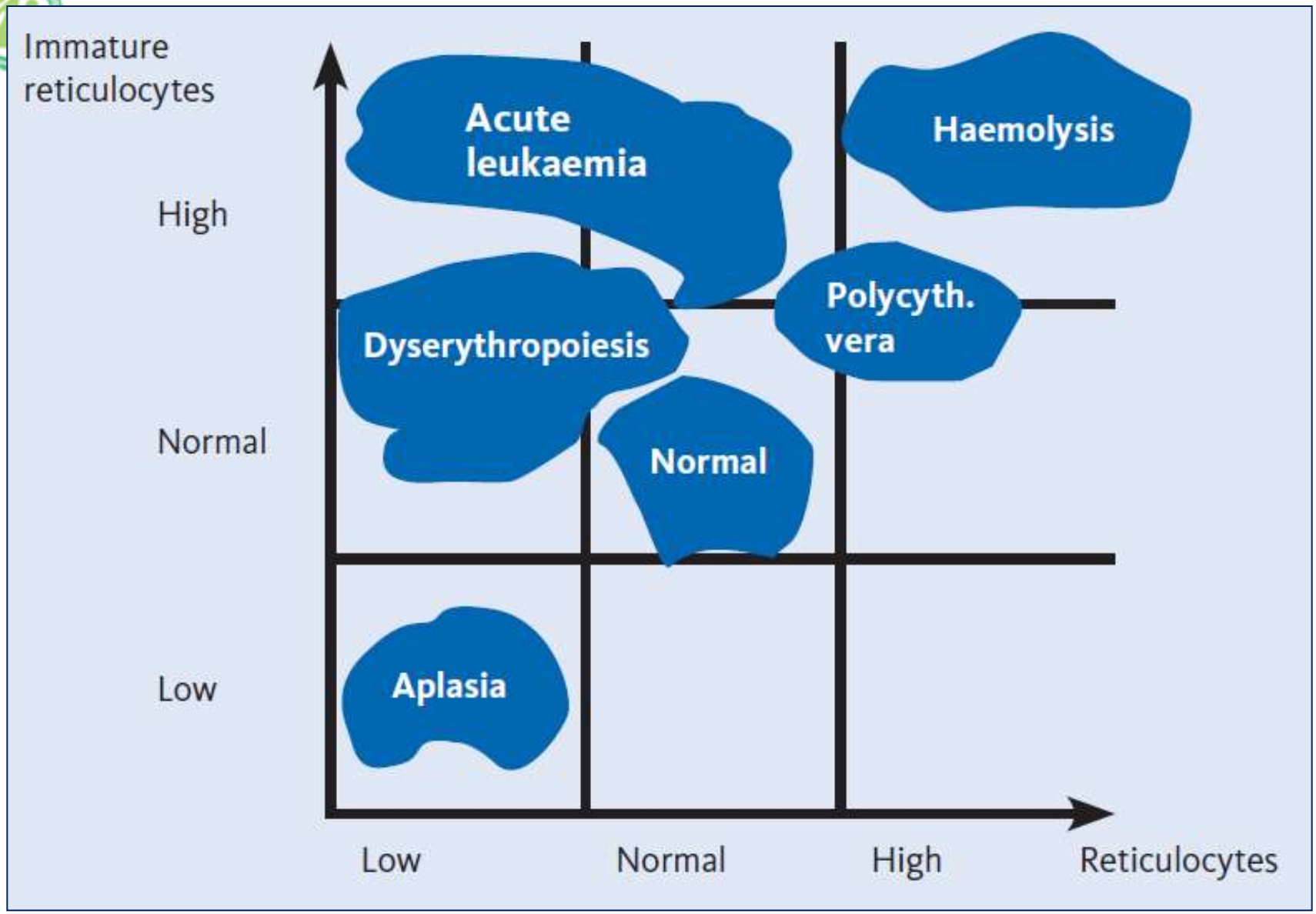
LFR	MFR	HFR
Low Fluorescence Reticulocytes	Medium Fluorescence Reticulocytes	High Fluorescence Reticulocytes
Little RNA	More RNA	High level of RNA
Mature reticulocytes	Semi-mature reticulocytes	Immature reticulocytes
Reference range: 86.5 - 98.5%	Reference range: 1.5 - 11.3%	Reference range: 0 - 1.4%

IRF = MFR + HFR

Reference range

IRF:	f	1.1 – 15.9 %
	m	1.5 – 13.7 %



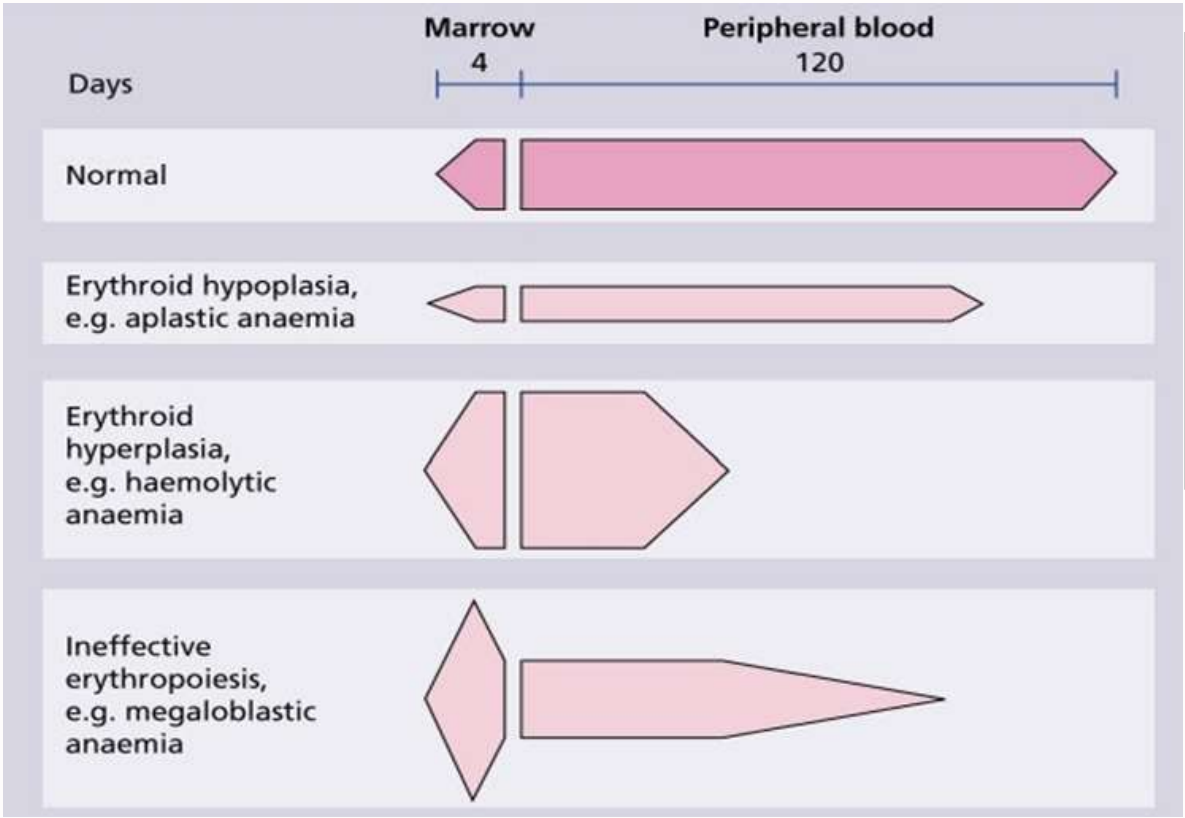


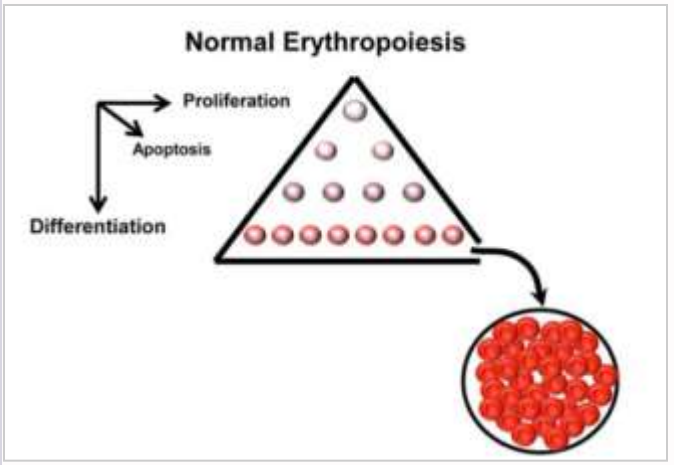
Reticulocytosis together with ↑ IRF is strongly indicative of hemolytic anemia

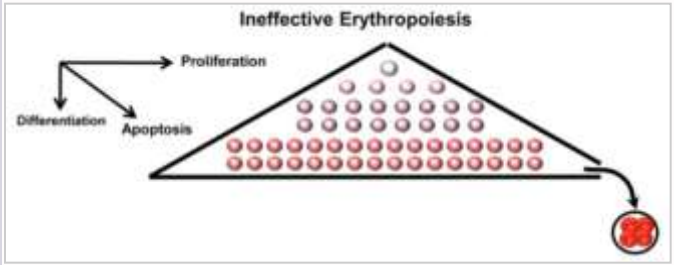
✓骨髓增生程度（Bone marrow cellularity）：反映骨髓红系增生状况

- 骨髓增生明显或极度活跃
- 粒红比例倒置（reversed GE ratio）

- 严重溶血情况下可发生溶血再障危象（hemolytic crisis）







Stepwise Diagnostic Approaches to Hemolytic Anemias

是否符合溶血性贫血诊断标准

- 临床表现的基础上，如出现RBC破坏增多和RBC代偿增生证据中的4~5项（其中必须包括RBC↓或Hb↓或Hematocrit↓），即可确定溶血性贫血的诊断
- 临床有时采用“排除法”确定有无溶血性贫血

Stepwise Diagnostic Approaches to Hemolytic Anemias

力争明确溶血性贫血的原因或基础疾病

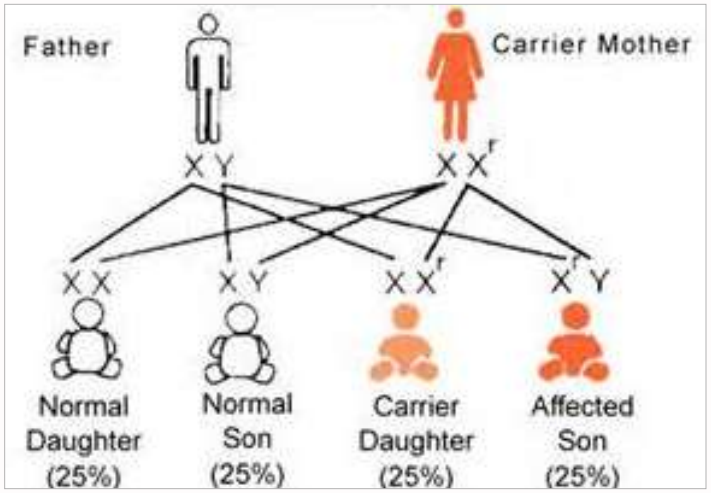
病史和体格检查

- 阳性家族史：G6PD缺乏症(X-连锁遗传），遗传性球形红细胞增多症（HS，常显遗传）和地贫(常隐遗传）等
- 人种和发病地区：黑热病，疟疾，地中海贫血等
- 诱因：红细胞酶病一般有诱因，如感染、药物和蚕豆等
- 典型临床表现
  - ✓ G6PD缺乏症：发作性急性贫血；黄疸；血红蛋白尿
  - ✓ 重型β地贫：发病早，进行性加重，地中海贫血面容和脾大
  - ✓ 药物相关性溶血性贫血：药物暴露史
  - ✓ 免疫性溶血性贫血：SLE相关性，可存在典型蝶形红斑

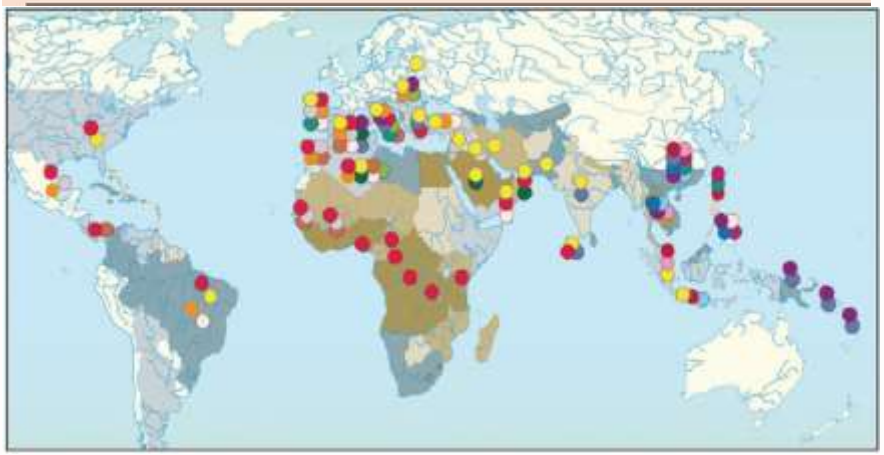
G6PD缺乏症

- G6PD缺乏为人类最常见遗传性红细胞酶缺乏症（enzymopathy），基因定位于Xq28。通常女性为携带者，男性发病

- 男性：正常半合子(Gd+) 或缺失半合子(Gd–)
- 女性：正常纯合子(Gd+/Gd+)、缺失纯合子(Gd-/Gd-)，或杂合子(Gd+/Gd-)。G6PD酶活性介于正常和纯合子之间

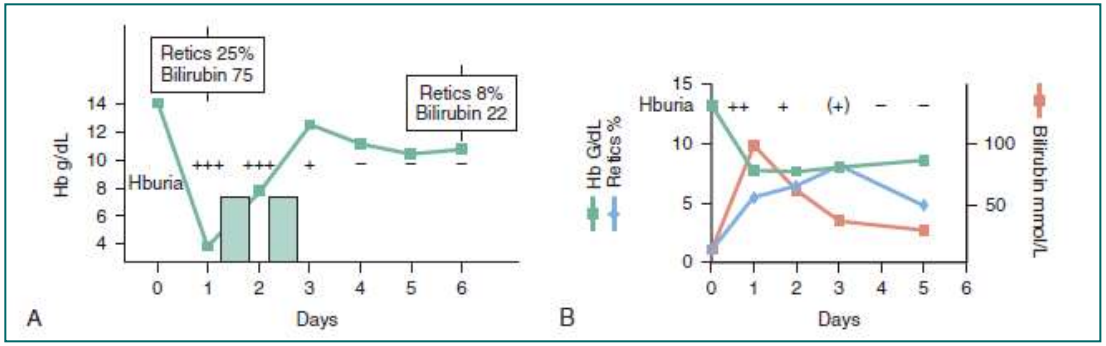


Class	Level of deficiency	Enzyme activity
I	Severe	Chronic nonspherocytic hemolytic anemia in the presence of normal erythrocyte function
II	Severe	Less than 10 percent of normal
III	Moderate	10 to 60 percent of normal
IV	Mild to none	60 to 150 percent of normal
V	None	Greater than 150 percent of normal

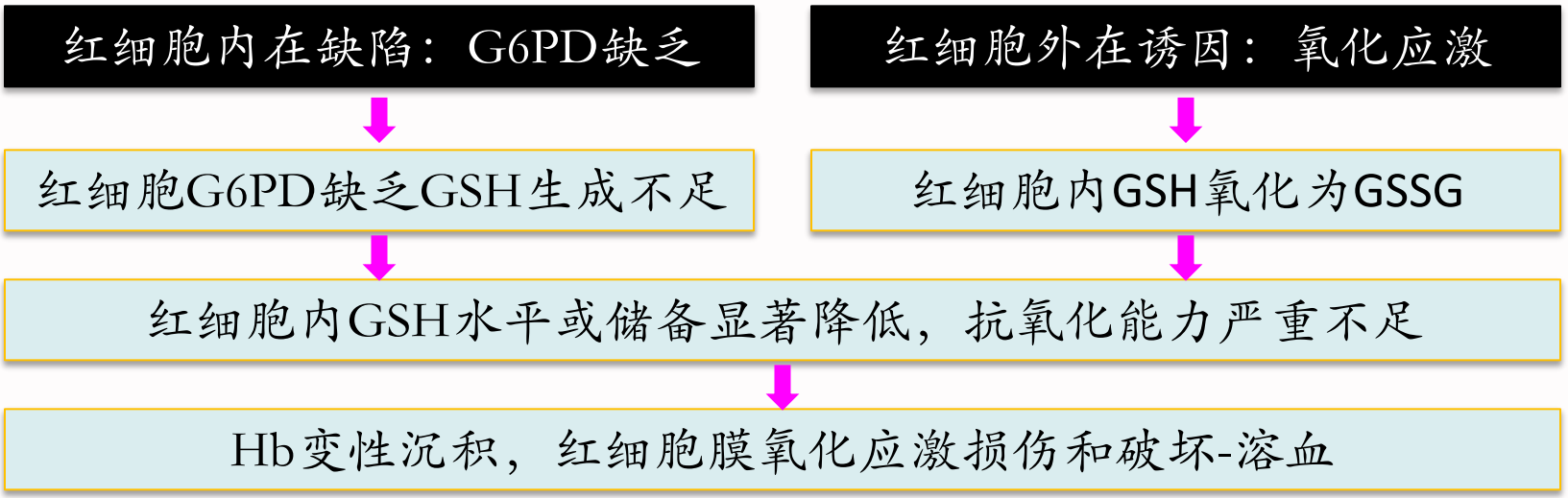


典型临床表现：急性溶血性贫血（蚕豆病为例）

- 平时无临床症状或表现，处于“稳态”(steady-state)
- 感染、服用蚕豆和氧化性药物/化学物暴露等诱因
- 急性起病：服用蚕豆后数小时后出现恶心、呕吐、腹痛和腹泻等消化道症状
- 血红蛋白尿：6~24小时内出现葡萄酒或酱油色小便
- 皮肤巩膜黄染，进行性面色苍白
- 体征：面色苍白、心动过速等。脾大少见。严重情况下休克或心力衰竭表现

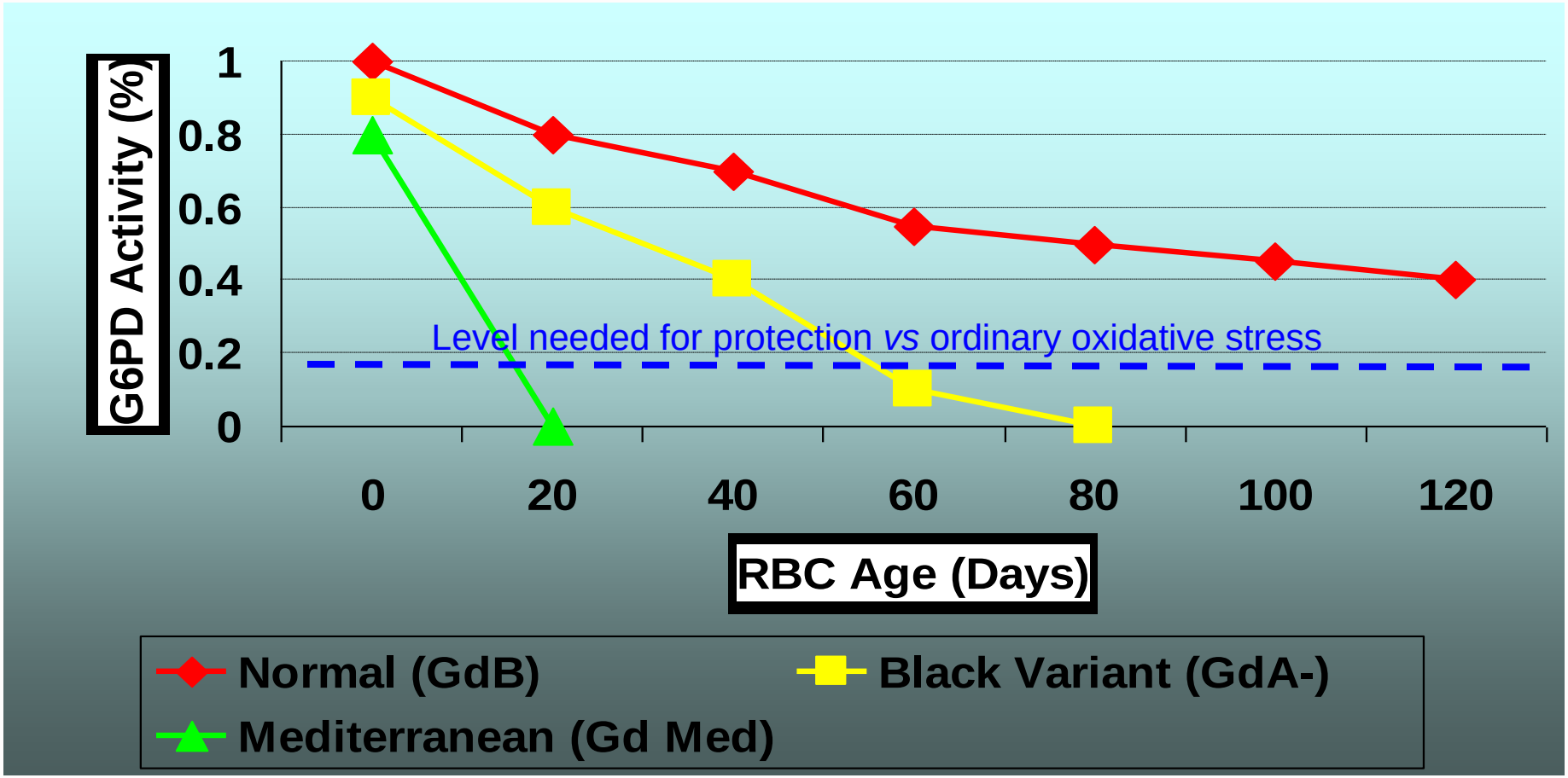


G6PD缺乏症的病理生理



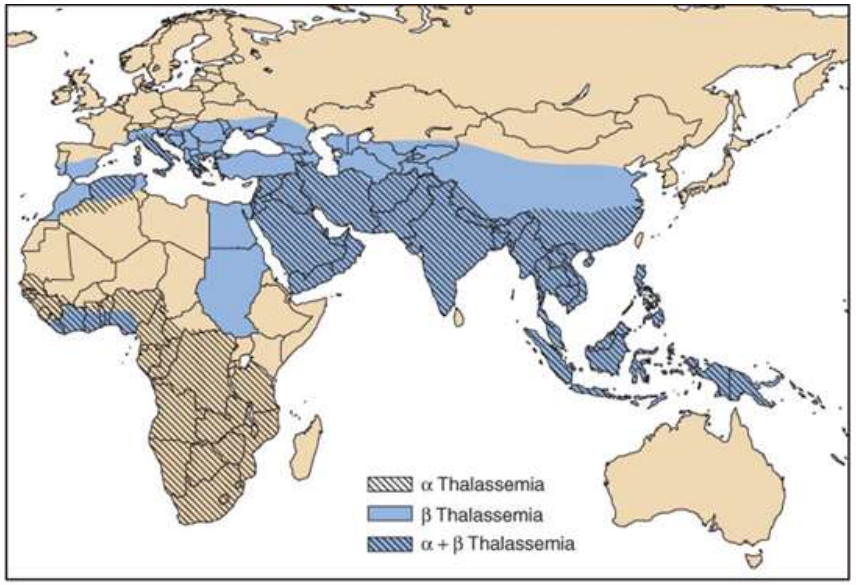
- **G6PD蛋白稳定性降低：** 最常见和最主要机制。氨基酸置换如累及亚单位结合部位，严重影响二聚体形成和蛋白稳定性，多表现为遗传性非球形红细胞性溶血性贫血（CNSHA）
- **G6PD酶催化功能降低：** 相对少见，可同时伴有蛋白稳定性降低

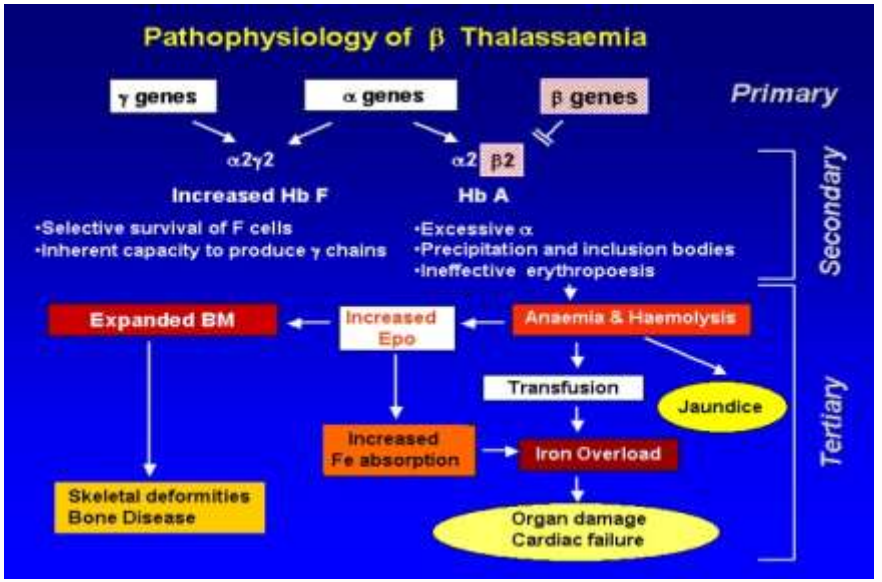
G6PD deficiency: genotype-phenotype correlations



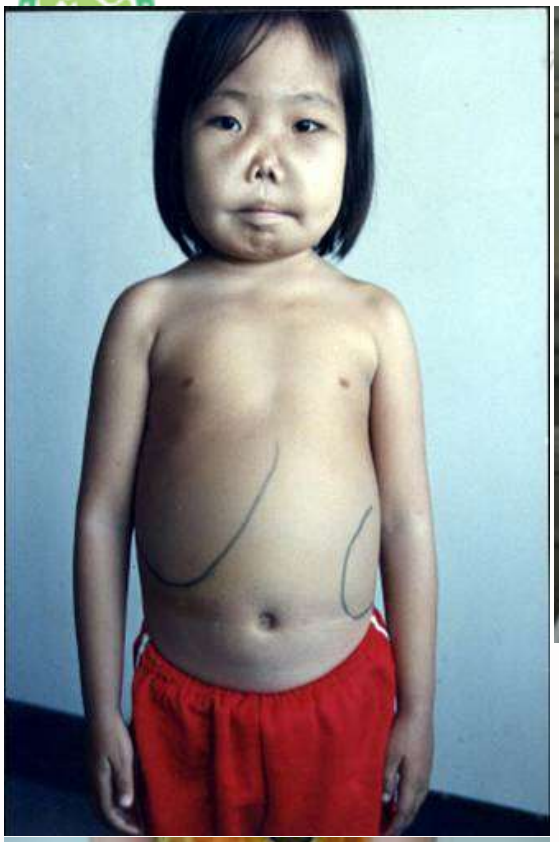
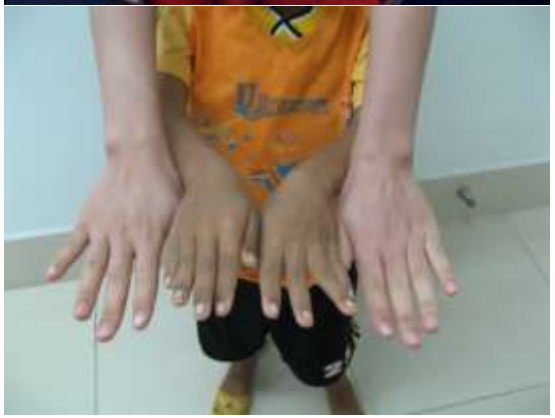
Gd^{B} 半衰期60 天；Gd^{A-}半衰期13天；Gd^{Med}半衰期5天

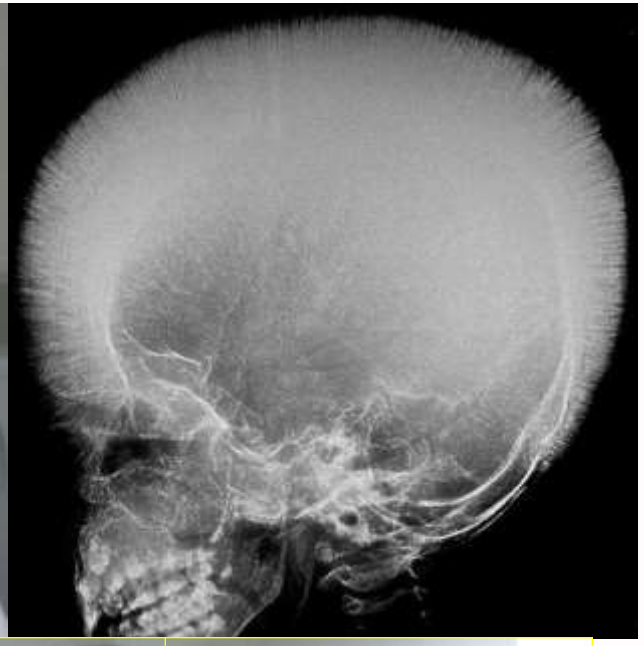
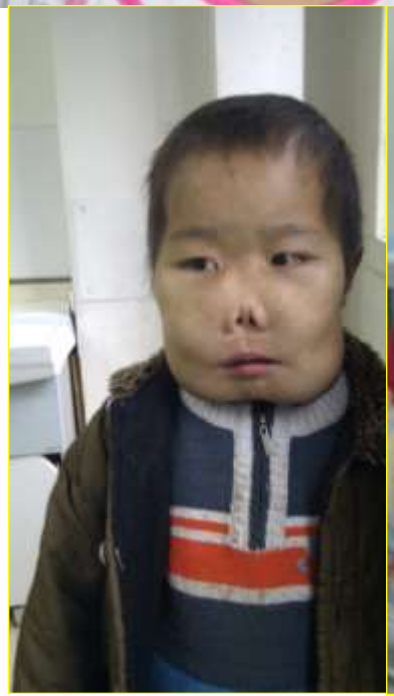
地中海贫血





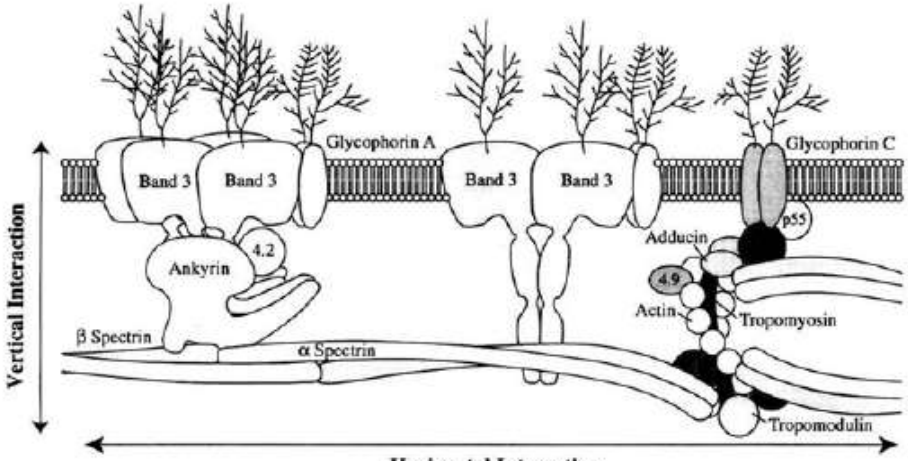
- 人类常见遗传性溶血性贫血，常隐遗传，呈世界性分布，我国以南方省市高发，阳性家族史有助于诊断，但阴性不能排除
- 典型的临床表现（重型β地贫）
  - 自幼起病（3-6月）；慢性进行性贫血，面色苍白，黄疸；肝脾肿大明显，腹部膨隆；地贫面容：颧骨隆起，眼距增宽，鼻梁低平；消瘦、体格发育落后；易感染、抵抗力差；继发性铁负荷增多相关并发症：肝硬化、糖尿病、性功能发育落后、甲状旁腺功能低下、尿崩症和垂体功能低下、顽固性心衰

遗传性球形红细胞增多症（Hereditary spherocytosis, HS)

- 红细胞膜骨架蛋白遗传缺陷所致，2/3病例常显遗传（ankyrin，band3和β-spectrin），部分呈常隐遗传（α-spectrin和band 4.2）。Ankyrin和spectrin联合缺陷最常见。 Band3和ankyrin联合缺陷临床表型最重



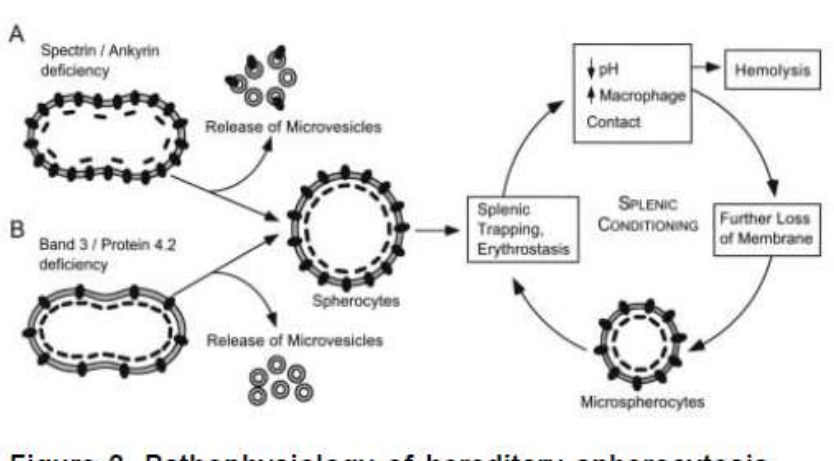


Figure 2. Pathophysiology of hereditary spherocytosis.

带3蛋白，蛋白4.2，锚蛋白或血影蛋白缺乏或功能异常，影响红细胞膜脂质双分子层和细胞骨架在垂直和水平方向的相互作用，导致两者解离，膜空泡化和丢失，导致红细胞表面积/体积之比降低和红细胞球形化，红细胞渗透脆性增加，最终被脾脏阻滞破坏导致溶血

Gallagher PG. Red cell membrane disorders. Hematology. 2005;2005:13-18.

典型临床表现

- 75%病例有阳性家族史（常显）
- 儿童期发病多见；新生儿期易发生黄疸（新生儿黄疸）
- 贫血、黄疸和脾脏肿大
- 外周血网织红细胞升高
- 球形红细胞增多
- 红细胞渗透脆性增加
- Coomb's试验阴性

球形红细胞增多症分类标准和切脾指针

分类指标	Trait	Mild	Moderate	Severe
Hb(g/L)	正常	110-150	80-120	60-80
Ret (%)	正常	3-6	>6	>10
胆红素 (μmol/L)	<17	17-34	>34	>51
Spectin (%)	100	80-100	50-80	40-60
切脾	无需	一般不	学龄至青春期	尽量6岁后

诊断试验(diagnostic testing)	推荐等级
☼ 具有阳性家族史、典型临床表现和实验室检查结果（球形红细胞增多、MCHC增加、Reticulocyte增加)的新诊断HS病例，无需其他实验室检查	1A
☼ 对不能确诊的病例，采用冷溶血试验(cryohemolysis)和伊红-5-马来酰亚胺 (eosin-5-maleimide, EMA)结合试验有助于诊断	1A
☼ 对不典型病例，可采用红细胞膜SDS-PAGE凝胶电泳分析	

Bolton-Maggs PH, et al. Br J Haematol. 2012;156(1):37-49.

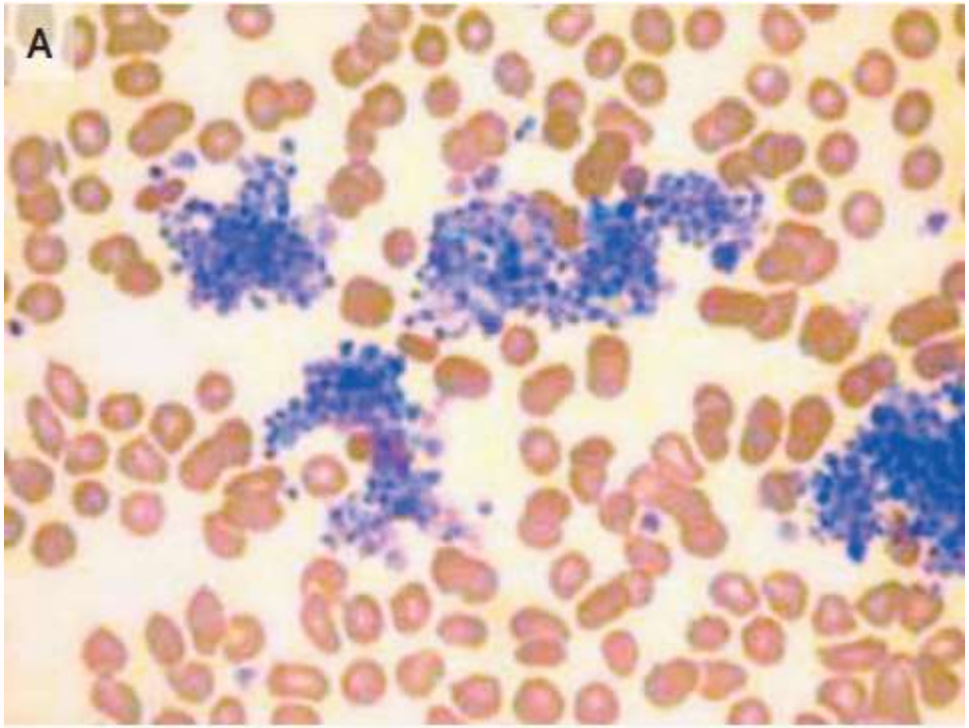
溶血尿毒综合征（hemolytic uremic syndrome, HUS）

- TMA（thrombotic microangioapthy）一组以微血管血栓形成和血流受阻、微血管病性溶血性贫血、血小板显著减少和不同程度器官缺血所致临床表现，包括HUS，TTP，HELLP综合征等
- HUS病因复杂，绝大部分与产生shiga toxin的肠道细菌感染有关（尤其是E coli O157:H17血清型，占70%），分为典型和非典型HUS（typical and atypical HUS）（或D+HUS和D-HUS）
- HUS三联症：微血管病性溶血性贫血（血红蛋白尿）、血小板减少和急性肾衰竭，婴幼儿和学龄期儿童多见，为ARF常见原因

Name	Clinical features
TTP	
Acquired TTP	Adults with or without neurologic or renal abnormalities; children without renal failure (with or without neurologic abnormalities); occurrence in children is rare
Hereditary TTP (Upshaw-Schulman syndrome)	Much less common than acquired TTP; caused by a mutation of the *ADAMTS13* gene resulting in a severe deficiency of ADAMTS activity; may present at any age: for example, newborn infants with severe hemolysis, children with recurrent thrombocytopenia, or women during their first pregnancy
HUS	
Typical HUS (90%-95% of children with HUS)	Children with renal failure who have preceding diarrhea, typically bloody, caused by Shiga toxin–producing *E coli* (almost always *E coli* O157:H7)
aHUS (5%-10% of children with HUS)	Children with renal failure who do not have preceding diarrhea; in 10% of children with aHUS, multiple family members are affected; the term aHUS is currently being used to describe patients with abnormalities of complement regulation, including both adults and children

典型病例（case illustration）

- 3岁男孩，因血小板减少拟诊为ITP入院
- 血小板减少，但血涂片发现“明显血小板聚集”
- 病史：前驱性腹泻、发热
- 进一步实验室检查：微血管病性溶血性贫血和肾功能损害
- Coomb试验阴性
- 诊断HUS
- 血浆置换治疗



Numerous platelet clumps (aggregates) resulting in factitious thrombocytopenia

自身免疫性溶血性贫血（autoimmune hemolytic anemia）

- 儿童常见溶血性贫血类型，病因复杂。5岁以下和男孩多见

Primary (idiopathic) AIHA

- Warm-Reactive (wAIHA)
- Cold Agglutinin Syndrome (CAS)
- Paroxysmal Cold Hemoglobinuria (PCH)
- Mixed-type

Secondary (associated) AIHA

- Systemic Lupus Erythematosus (SLE)
- Chronic Lymphocytic Leukemia (CLL)
- Evans Syndrome (ES) – AIHA + ITP
- Drug induced
- Post-infection

Michel M. *Expert Rev Hematol*. 2011 Dec;4(6):607-18.

- ✓ 原发性：37%
- ✓ 感染后：10%
- ✓ 自身免疫疾病：53%

- ✓ 温抗体型：70%-80%
- ✓ 冷抗体型：15%-20%
- ✓ 混合型：10%

- 前驱性感染、药物暴露、自身免疫疾病
- 发热，面色苍白，乏力，黄疸，血红蛋白
- 部分病例肝脾肿大
- 贫血、reticulocytosis，Coomb's阳性

发病机制

Idiopathic formation of Ab to erythrocytes

- May be secondary to autoimmune disease, infection or malignancy
- IgG in wAIHA, IgM in CAS

Ab binding to erythrocyte antigens

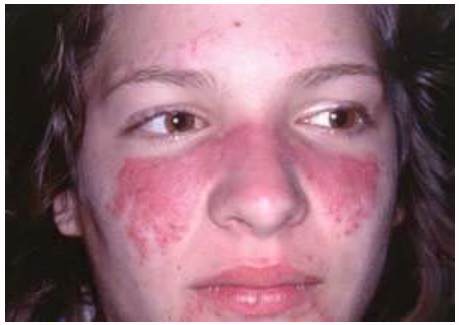
- 37°C in wAHIA, 4°C in CAS
- Complement fixation: IgM > IgG

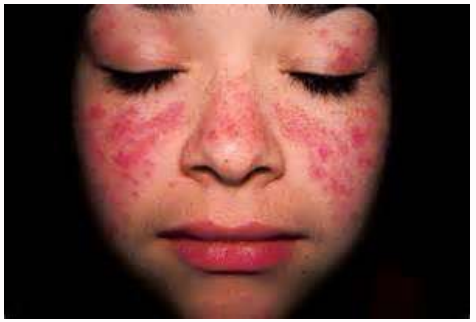
Erythrocyte destruction

- wAHIA: by macrophage ingestion in spleen
- CAS: by macrophage ingestion in liver + intravascular hemolysis

典型病例（case illustration）

- 12岁女孩，因全血细胞减少入院
- 拟诊“再生障碍性贫血”
- 骨髓提示增生明显活跃
- Coomb’s试验阳性
- 自身抗体检测：ANA滴度1:3000
- 激素和CTX治疗





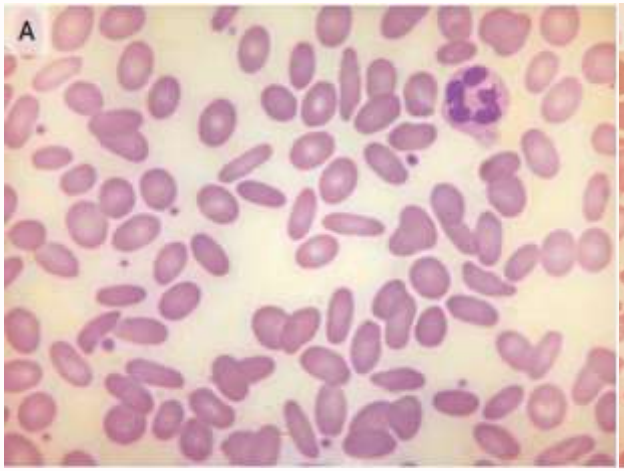
Butterfly typically found in SLE

试验室基线检查（Baseline Lab Investigation）

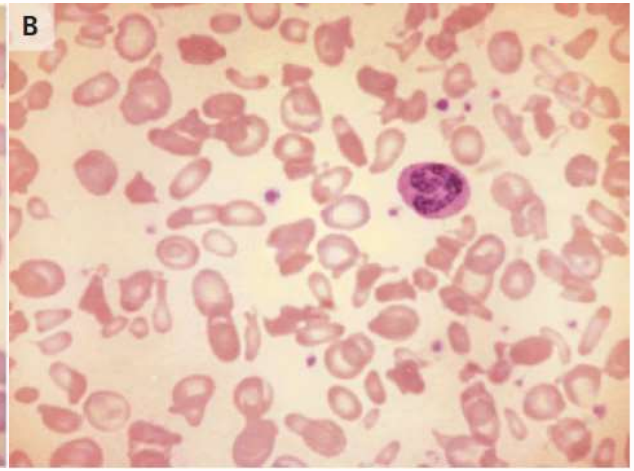
- 溶血性 贫血诊断和明确病因的重要依据
- 结合病史和体检合理选择

- **血液常规**
  - Baseline examinations，尽管非特异性，可提供重要诊断线索
  - 贫血：程度不一，多为正细胞正色素贫血，或大细胞性贫血
  - Reticulocytosis为溶血性贫血的共同特征和重要诊断依据
  - 重视WBC和分类计数：CLL易于合并溶血性贫血
  - 重视有无血小板异常：贫血伴血小板减少，重点考虑免疫性（如SLE，Evens综合征）和TMA

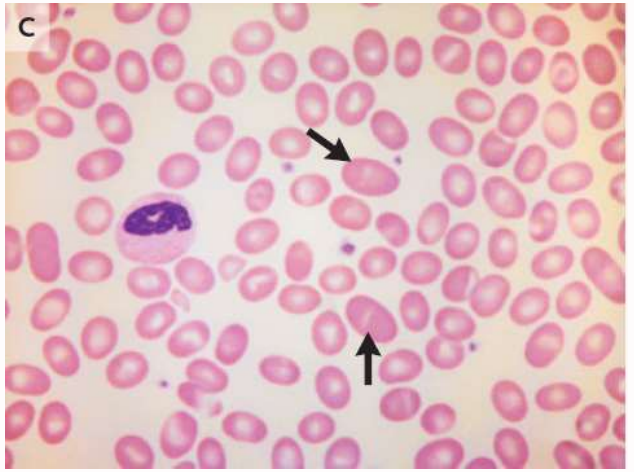
- 血液涂片：贫血（溶血性贫血）重要诊断线索
- 溶血性贫血外周血易见多种异性红细胞（poikilocytes）



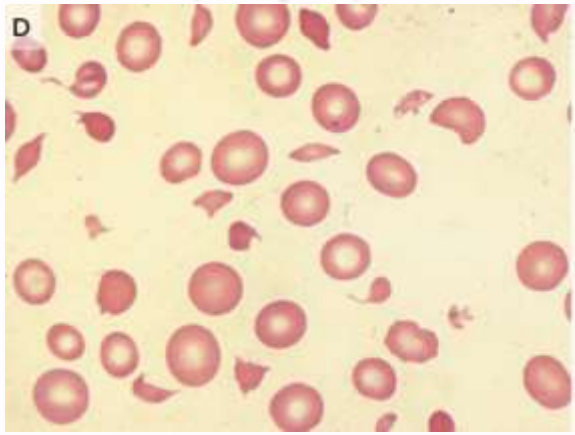
Hereditary Elliptocytosis(HE)



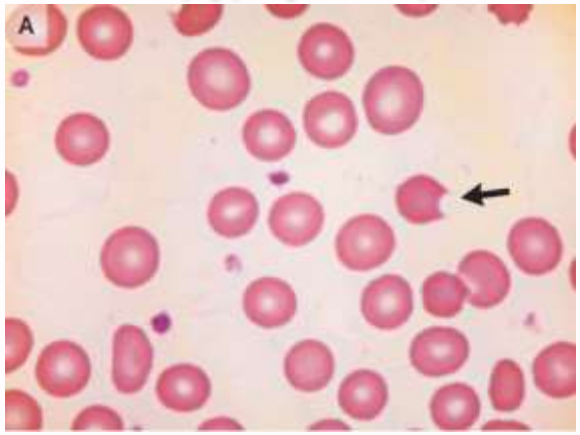
Hereditary pyropoikilocytosis



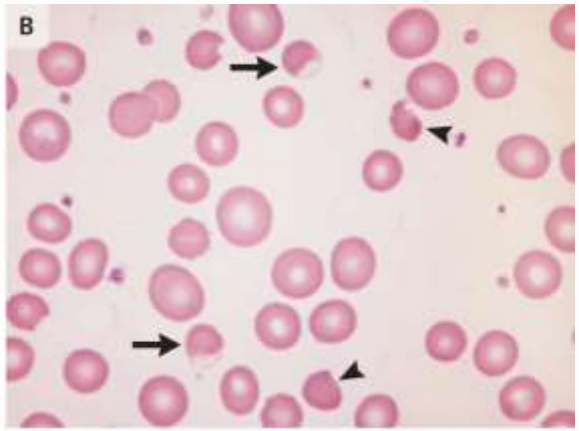
South East Ovalocytosis



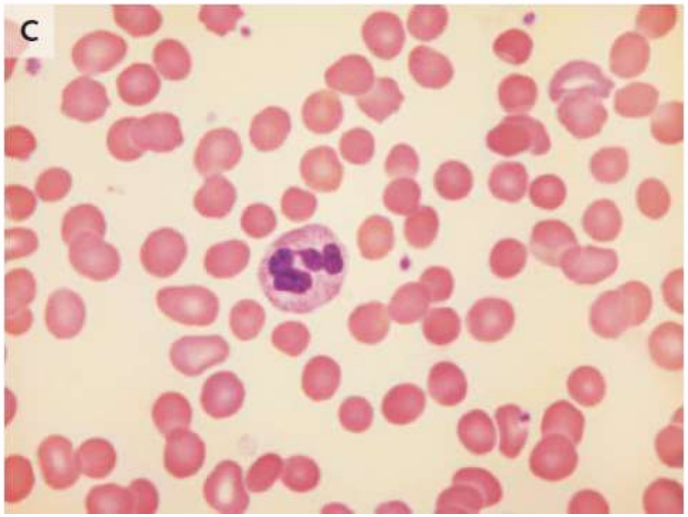
Red cell fragments in TMA



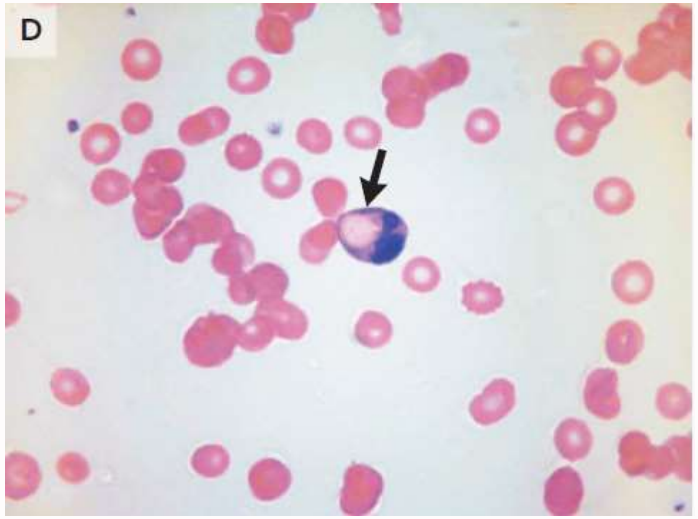
Bite cell seen in G6PDD



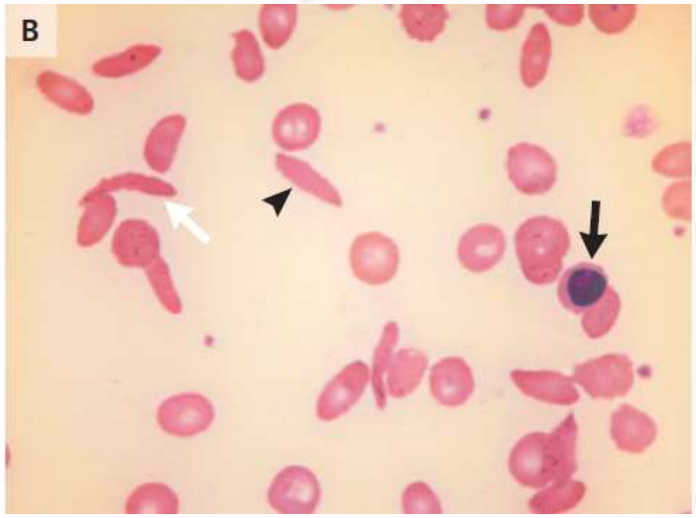
hemighosts in G6PDD



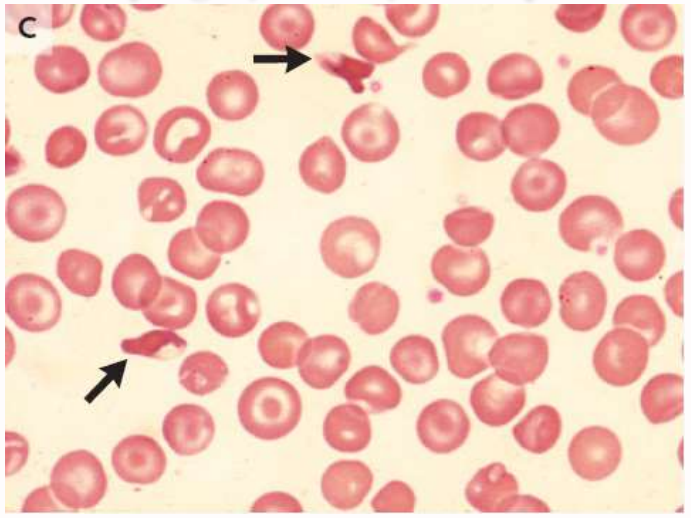
Numerous spherocyte in HS



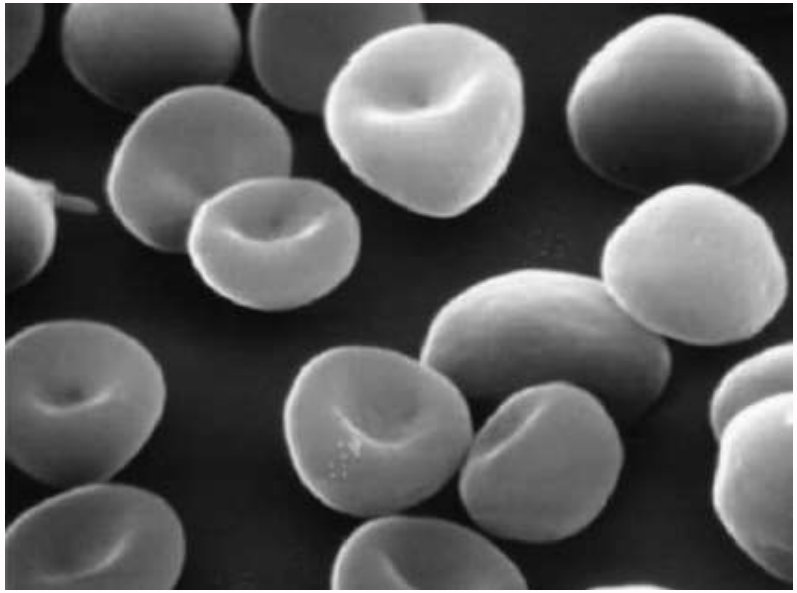
Red cell phagocytosed by neutrophil in PCH



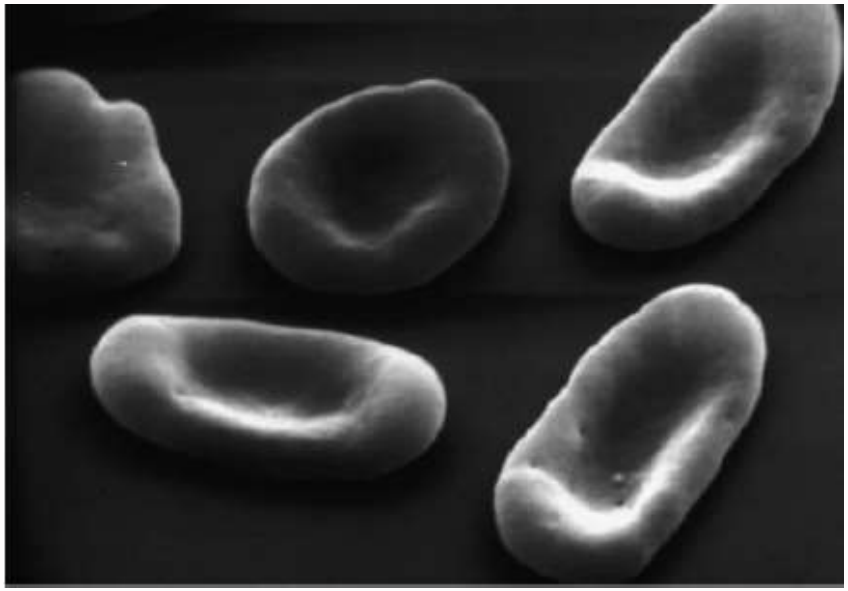
Nucleated red cell, sickle cells, and boat-shaped cells in sickle cell anemia (SCA)



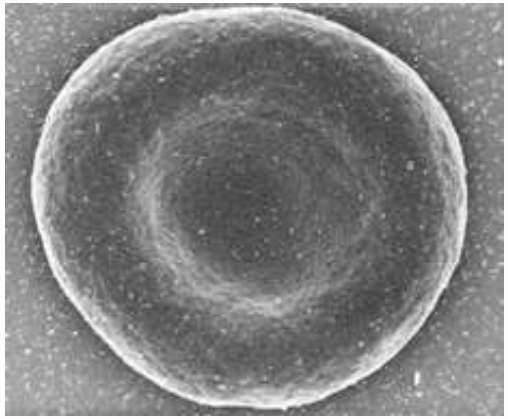
Target cells in thalassemia

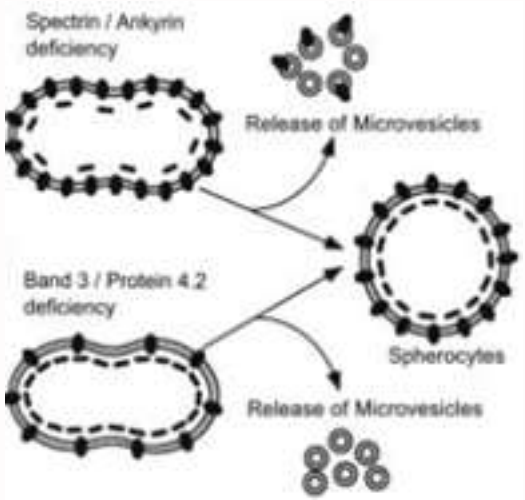


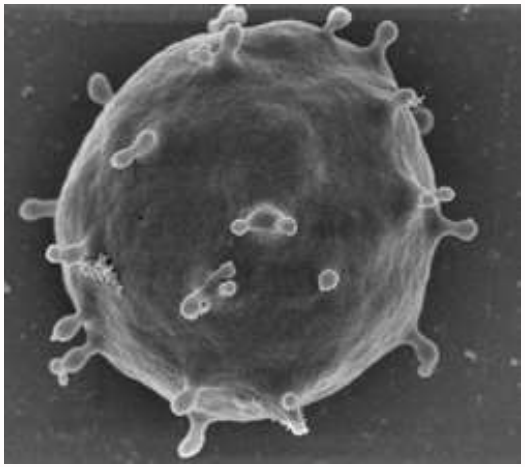
Hereditary Spherocytosis (HS)

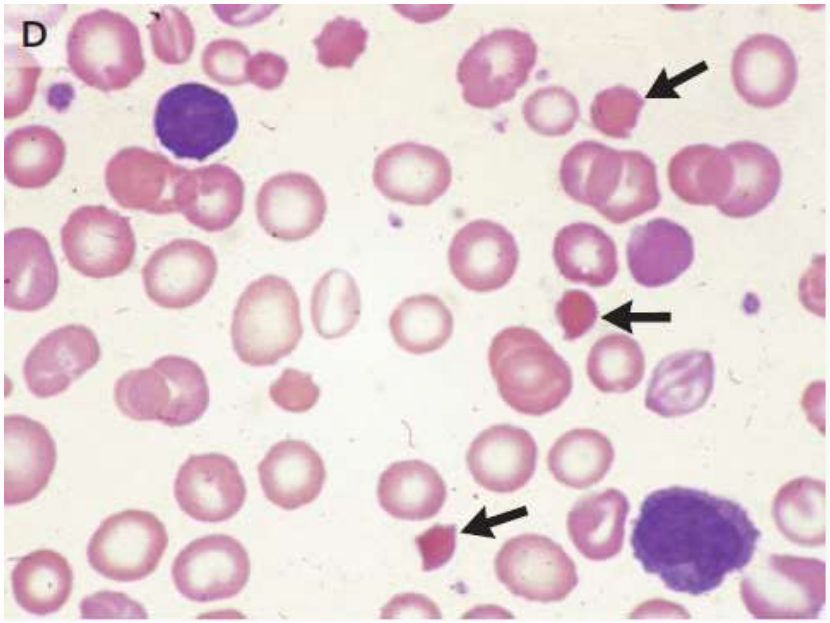


Hereditary Elliptocytosis (HE)

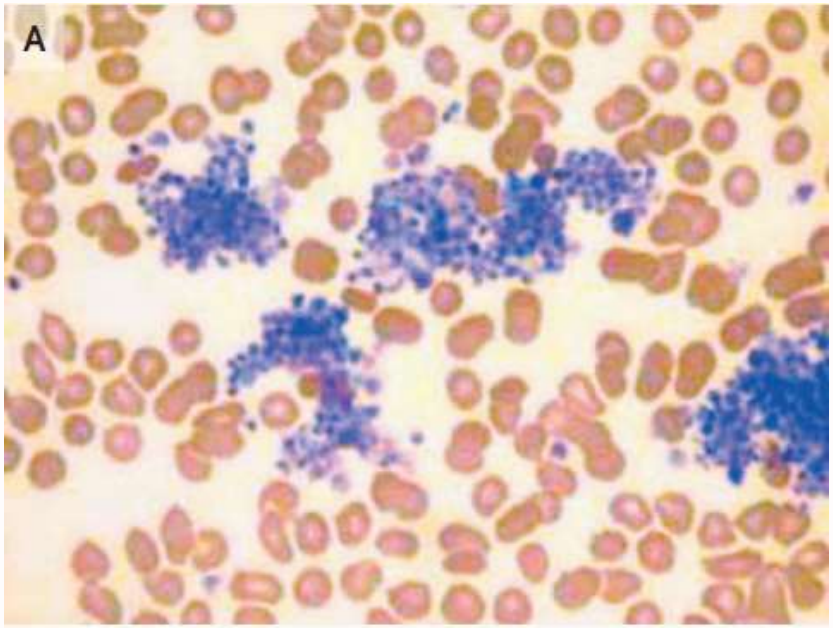




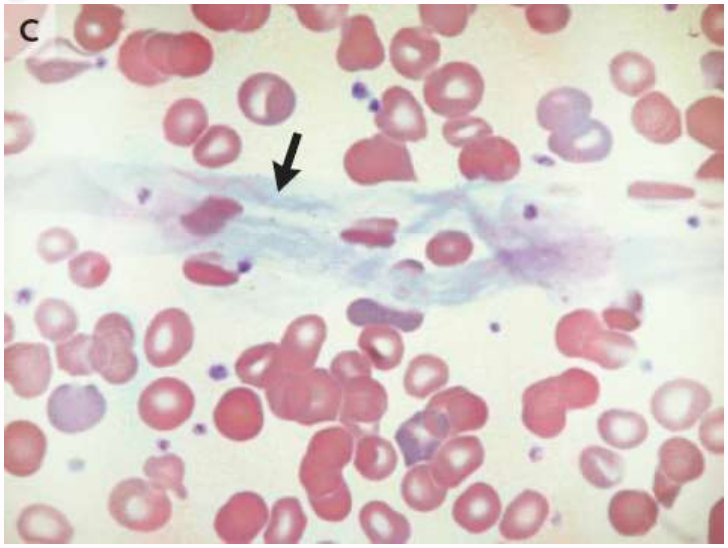




Irregular contracted red cells in unstable Hb Disease

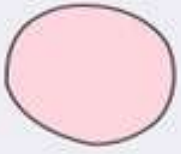


Platelet clumping in TMA



Fibrin strands documented in TMA

Bain BJ. Diagnosis from the blood smear. New Engl J Med. 2005; 353:498-507.

	Red cell abnormality	Causes
	Normal	
	Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia
	Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy
	Stomatocyte	Liver disease, alcoholism
	Pencil cell	Iron deficiency
	Echinocyte	Liver disease, post-splenectomy. storage artefact
	Acanthocyte	Liver disease, abetalipo-proteinaemia, renal failure

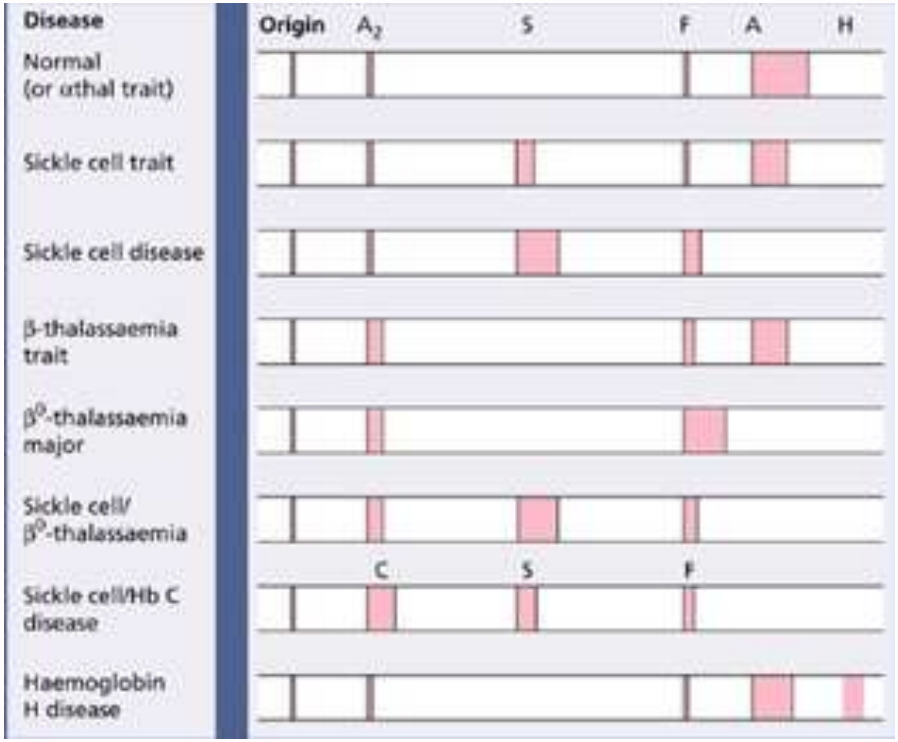
	Red cell abnormality	Causes
	Microspherocyte	Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia
	Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
	Elliptocyte	Hereditary elliptocytosis
	Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
	Basket cell	Oxidant damage– e.g. G6PD deficiency, unstable haemoglobin
	Sickle cell	Sickle cell anaemia
	Microcyte	Iron deficiency, haemoglobinopathy

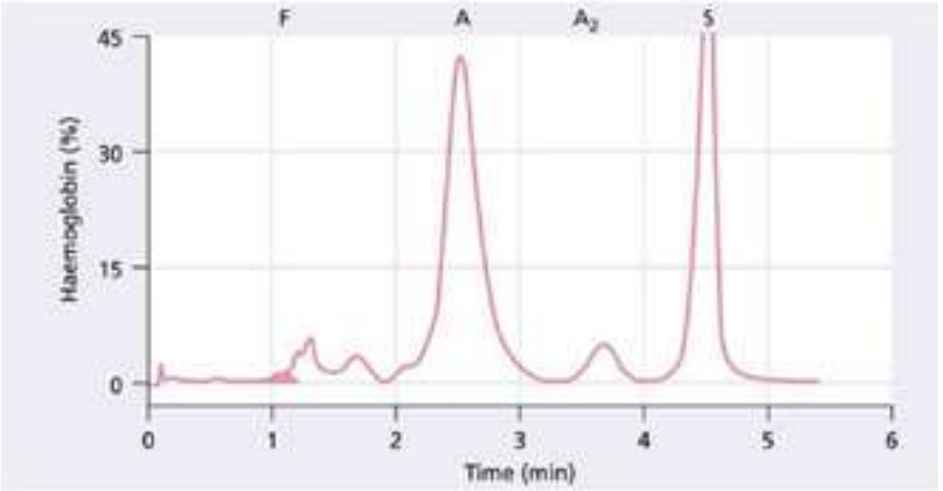
特殊试验室检查（Specific Lab Investigation）

溶血性 贫血诊断的重要依据。结合病史和体检合理选择

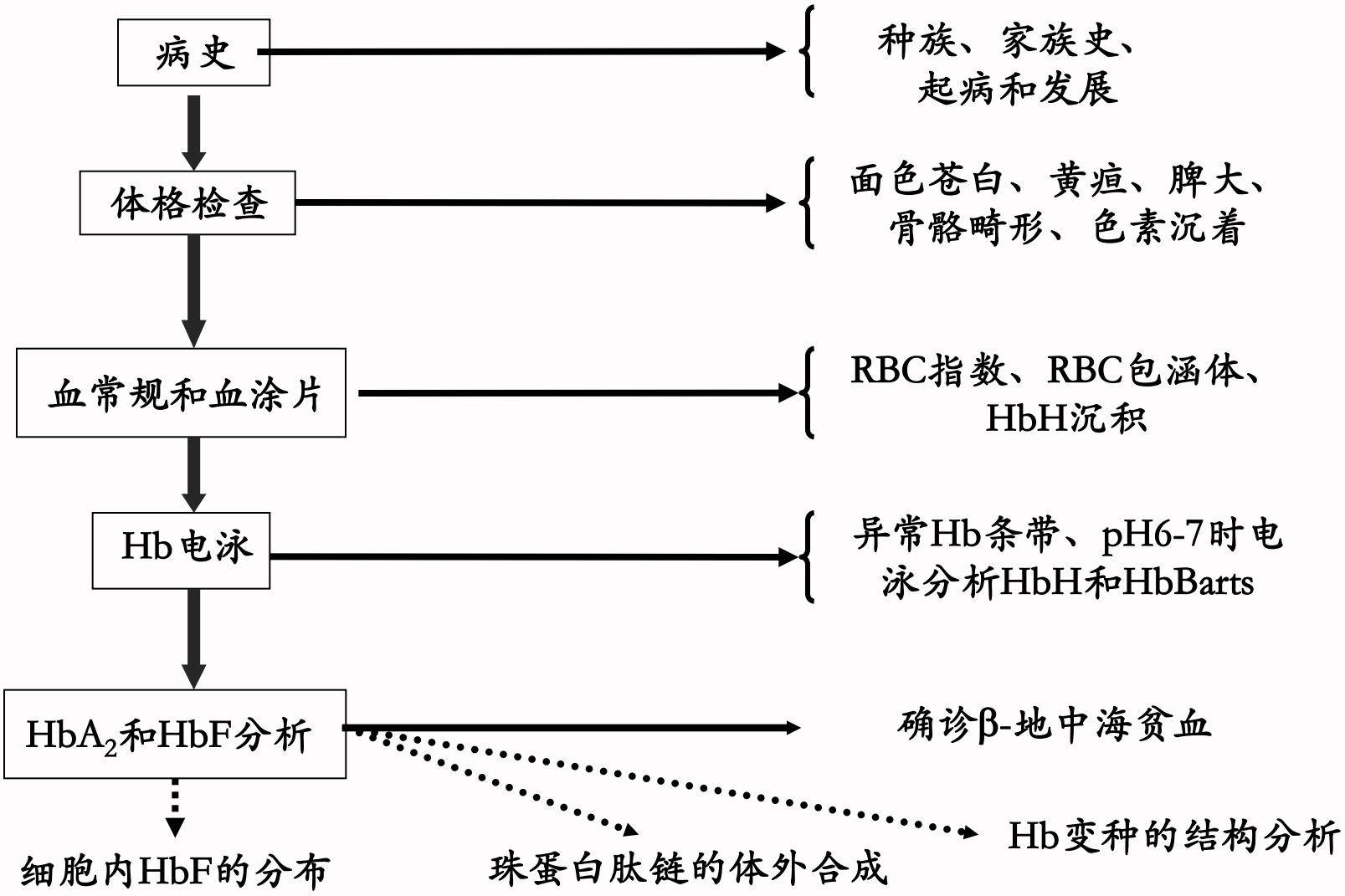
- G6PD缺乏症-G6PD酶活性测定，包括筛选试验和定量测定。
- 目前多推荐定量分光光度法（quantitative spectrophotometric assay）测定红细胞NADPH生成速率作为确诊G6PD活性的依据，WHO推荐Zinkham法。荧光斑点试验（fluorescent spot test）、高铁血红蛋白还原试验、硝基四氮唑蓝纸片法等半定量方法为筛查试验。无论初筛试验或定量试验均难以确诊女性缺失杂合子。正常参考值：6-10 IU/gHb
- G6PD活性正常或增高：急性溶血后网织红和年轻红细胞比例增高；输血

- 地贫-Hb电泳；高效液相色谱、等电位聚焦和基因突变检测

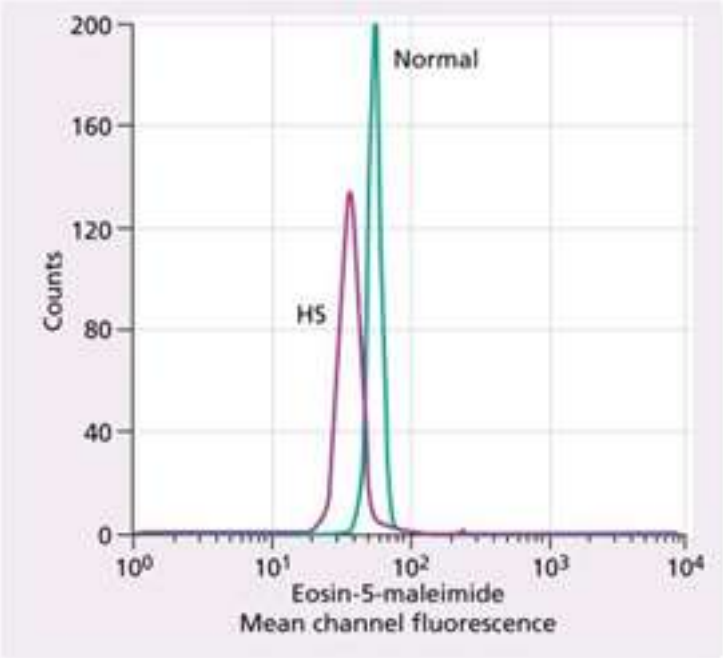




地中海贫血的诊断流程图



- HS：（孵育）红细胞渗透脆性试验、伊红-5-马来酰亚胺(eosin-5-maleimide, EMA)结合试验，冷溶血试验，SDS-PAGE，基因突变检测



诊断试验(diagnostic testing)	推荐等级
☼ 具有阳性家族史、典型临床表现和实验室检查结果（球形红细胞增多、MCHC增加、Reticulocyte增加)的新诊断HS病例，无需其他检查	1A
☼ 不能确诊的病例，采用冷溶血试验(cryohemolysis)和伊红-5-马来酰亚胺 (eosin-5-maleimide, EMA)结合试验有助于诊断	1A
☼ 对不典型病例，可采用红细胞膜SDS-PAGE凝胶电泳分析	

HS与其他遗传性红细胞膜缺陷的鉴别诊断

诊断	OF-test	Acid glycerol lysis-time test	Osmotic gradient ektacytometry	Cryohemolysis	EMA binding test
HS	↑	缩短	Distinct HS profile	↑	荧光降低
AIHA	↑	缩短	Similar to HS	?	正常或↑
Hereditary pyropoikilocytosis	?	?	?	?	↓↓
Overhydrated HSt	↑	?	Distinct profile	?	↑
Cryohydrocytosis	?	?	Distinct profile	?	↓
CDA-II	↑	?	?	正常或↑	正常或↓
SAO	?	?	不变形	↑	↓

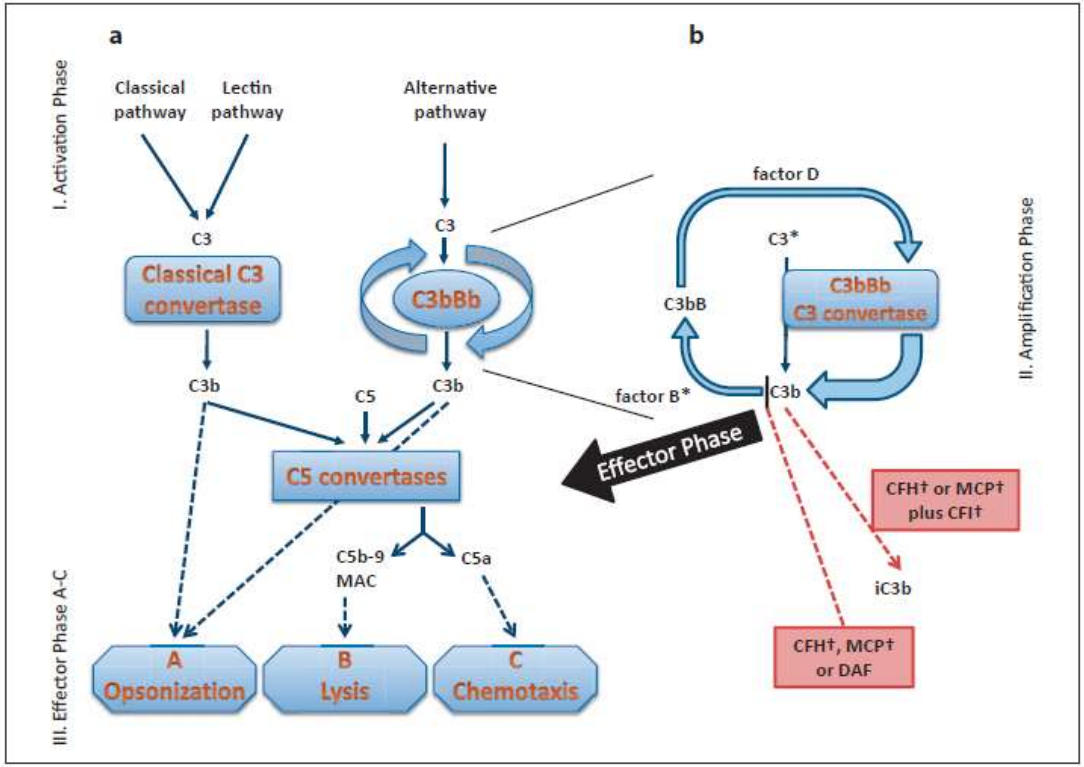
- 10%-20%HS患者OF正常英国血液学会标准委员会不再推荐常规渗透脆性试验（Osmolarity fragility，OF）
- FCM检测EMA与特定红细胞膜蛋白结合产生的荧光强度反映膜骨架蛋白水平，推荐为HS初筛试验，快速简便，敏感性和特异性与其他试验（如酸化甘油溶血试验和ektacytometry）相当，优于渗透脆性试验。也有助于HS与遗传性口形细胞和棘形细胞增多症(pyropoikilocytosis)的鉴别。
- 酸化甘油溶血试验（acidified glycerol lysis test, AGLT）较之渗透脆性试验阳性率更高，但特异性不好

补体旁路异常活化为不典型HUS重要发病机制

- Absence of Shiga toxin-producing bacteria as a triggering factor
- Abnormal activation of complement alternative pathway， primarily resulting from loss-of-function mutations of CFH, factor I (CFI) and membrane cofactor protein (MCP), or due to CFH deficiency caused by anti-CFH antibodies

Gene	Location	Frequency in aHUS, %
CFH	RCA gene Chr 1	10–30
MCP	RCA gene Chr 1	10–15
CFI	Chr 4	5–10
CFB	Chr 6	0–3
C3	Chr 19	N/A

Hirt-Minkowski P, Dickenmann M, Schifferli JA. Atypical hemolytic uremic syndrome update on the complement system and what is new. Nephron Clin Pract. 2010;114(4): C219-235.



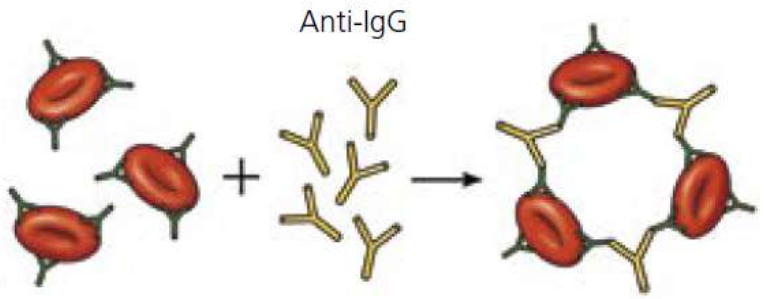
- aHUS: 包括筛选试验和定量测定

Table 1. Diagnostic tests recommended in patients presenting with the clinical features of aHUS

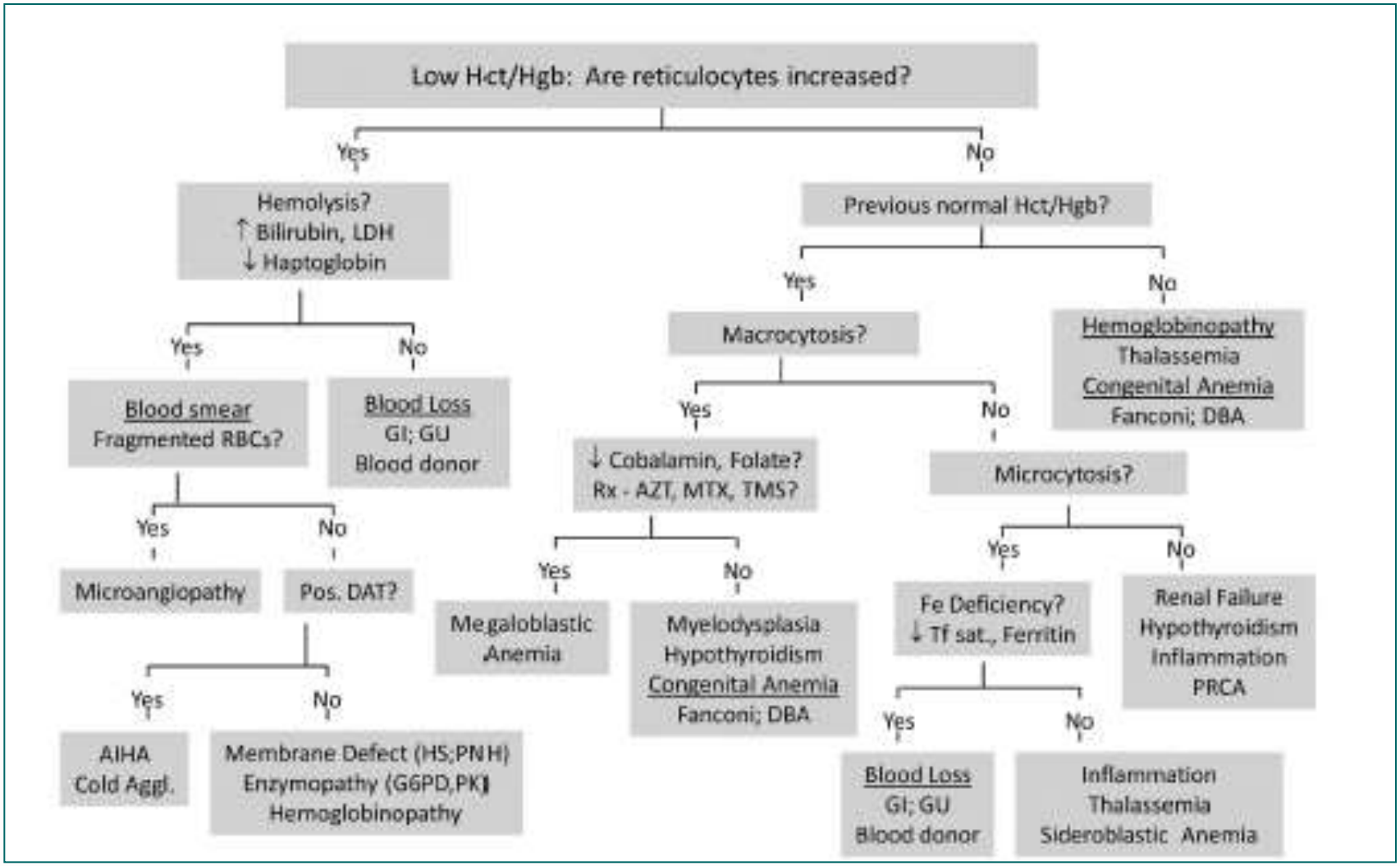
- Serum levels of C3, C4, factor H, and factor I
- Measurement of MCP expression on PBMCs by FACS
- Mutation screening of *CFH*, *CFI*, *CD46*, *CFB*, *C3*, and *THBD*
- Screening for genomic disorders affecting *CFH* and *CFHRs* 1-5
- Screening for factor H autoantibodies
- Measurement of ADAMTS13 activity

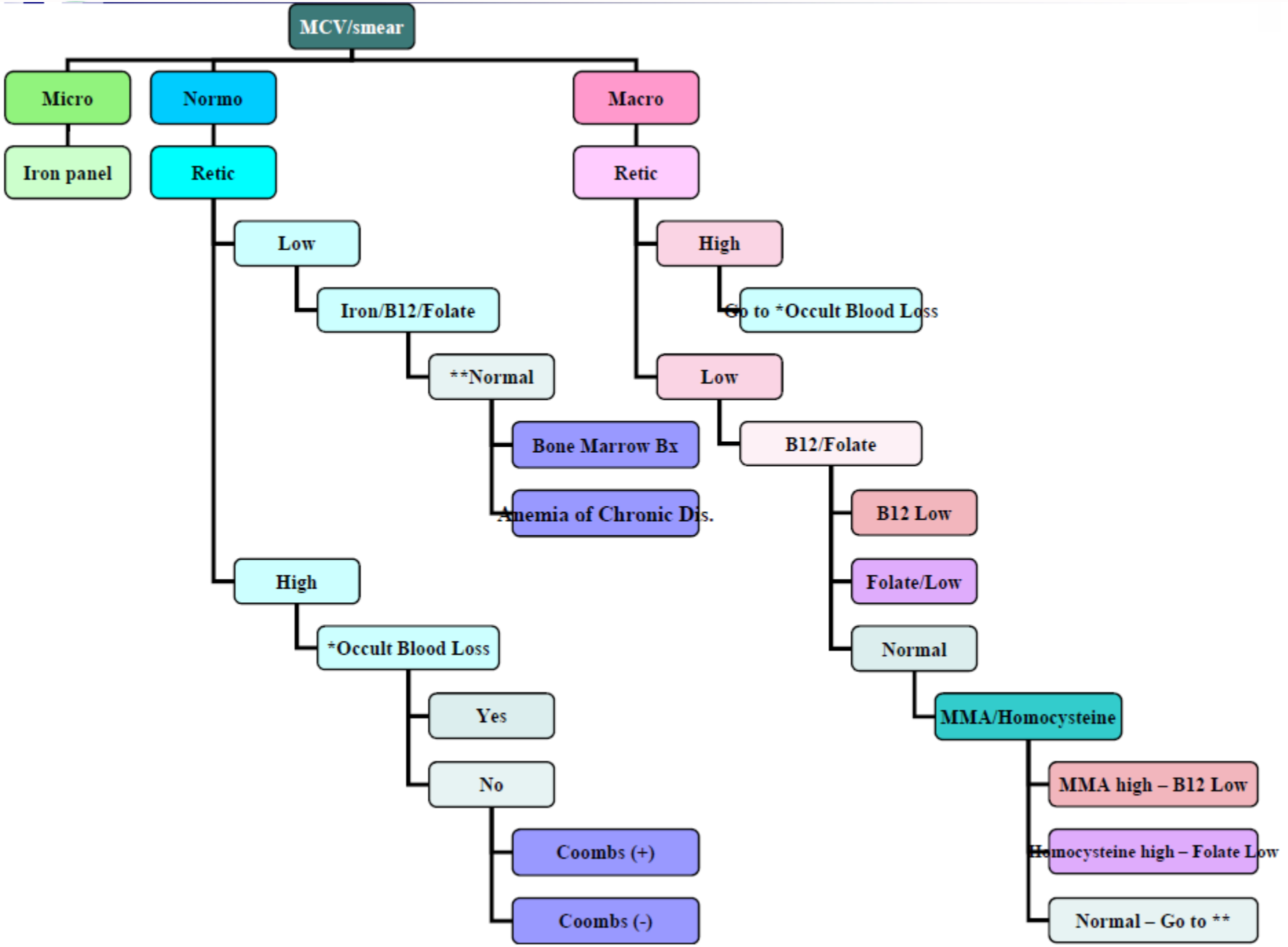
自身免疫性溶血性贫血

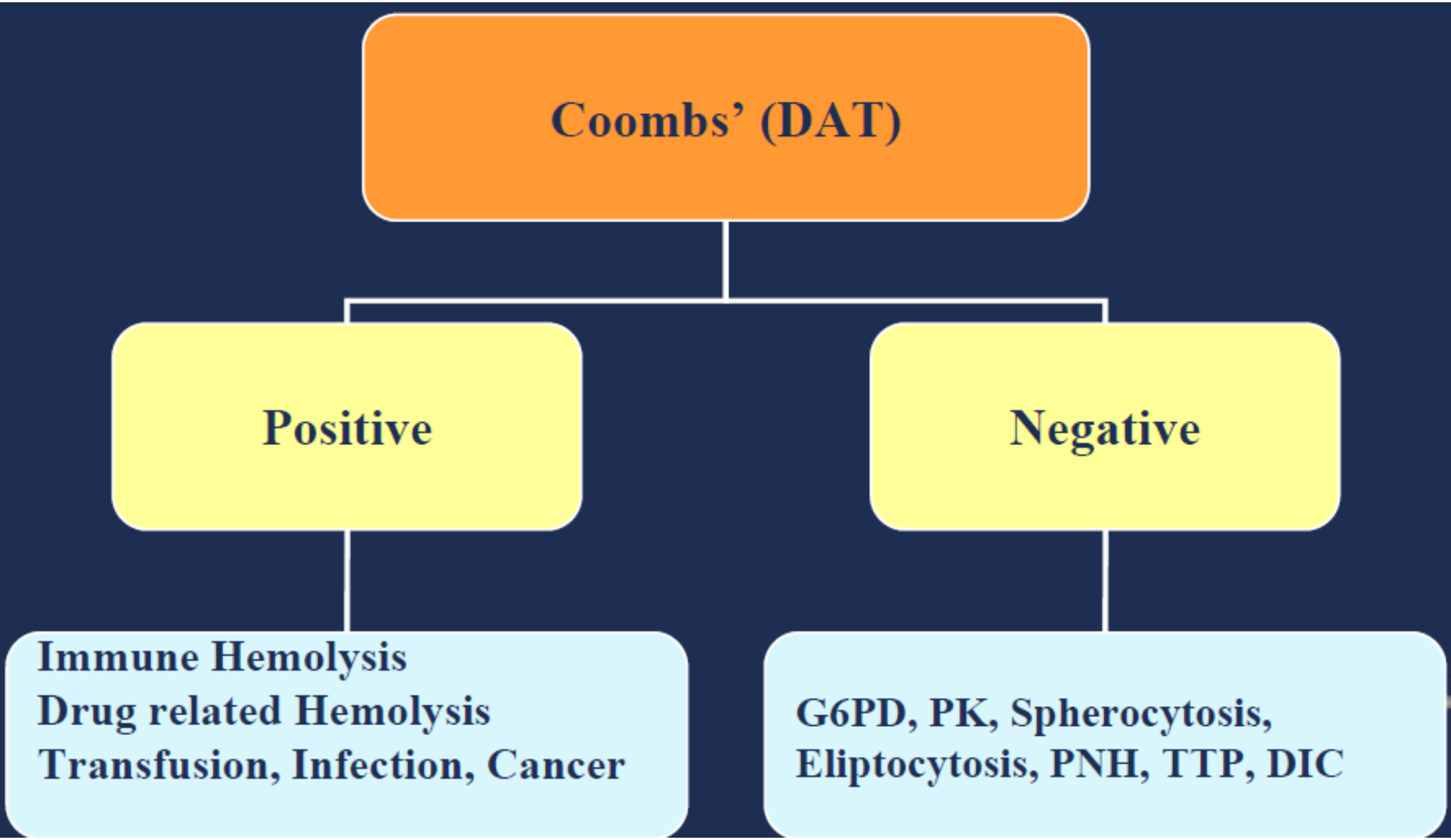
1. Direct Antiglobulin Test (DAT, direct Coomb's test)
   - detects IgG or complement on patient's red cells
   - the vast majority of patients with active immune hemolysis will have a positive DAT.
2. Indirect Antiglobulin Test (IAT, indirect Coomb's test)
   - detects antibody in patient's serum against red cell antigens
   - A positive IAT does not necessarily mean hemolysis is occurring - It may simply mean allo-immunization due to previous exposure to "foreign" red cell antigens (past pregnancy or transfusion).
3. Peripheral Blood Film: spherocytes



诊断流程（Diagnostic Approaches）




MCV/smear
Micro
Normo
Macro
Iron panel
Retic
Retic
Low
High
Iron/B12/Folate
Go to *Occult Blood Loss
**Normal
Low
Bone Marrow Bx
B12/Folate
Anemia of Chronic Dis.
B12 Low
High
Folate/Low
*Occult Blood Loss
Normal
Yes
MMA/Homocysteine
No
MMA high – B12 Low
Coombs (+)
Homocysteine high – Folate Low
Coombs (-)
Normal – Go to **



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Thank You for Your Attention