Sickle Cell Disease: diagnostic approaches and challenges

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Sickle Cell Disease results from a single mutation: HBB: c.20A>T (p.Glu7Val) $\Leftrightarrow$ Haemoglobin S

courtesy of Henri Wajcman
SCD: a group of disorders caused by different genotypes — all including the βS gene

Several common and some rare genotypes

– Sickle cell anemia (SCA = SS disease)
– HbSC disease
– HbS-beta thalassemia (β° or β+)
– HbSD\textsuperscript{Punjab}, HbS/O\textsuperscript{Arab}, HbSE
– Rare dominant variants S\textsuperscript{Antilles}, S\textsuperscript{Oman}
  (double mutation)

Considerable clinical heterogeneity between but also within each SCD genotype
Distribution of SCD

More than 400,000 newborns/year
(80% low-income countries)
Carriers represent 5% of the world population
Distribution of SCD

SCD is not a disease of the black people only
Hb S [β6 (A3)Glu→Val]

The diagnosis is phenotypic
Conventional electrophoreses or HPLC

Conventional electrophoreses

Isoelectric focusing (IEF)

Capillary electrophoresis

High performance liquid chromatography (HPLC)
Limits of the phenotypic diagnosis

Compound heterozygotes
  HbS/β-thal
  β°-thal/β⁺-thal
  HbS/rare Hb variant

Father not available

Rare variants with clinical expression
  HbS Antilles / HbS Oman

Prenatal diagnosis

Preimplantation diagnosis

⇒ DNA testing
New developments in SCD testing

- Point of Care (PoC) tests
- High-throughput newborn screening platforms
**PoC tests**

**Immuno essais**

**SickleScan™**

**Goals:**

- no specific equipment
- result in less than 15 minutes
- by a trained but unqualified personnel
- cost lower than 1 € (at present 5 €)
PoC tests

Immuno essais

Missing line = positive result

HemoTypeSC™
PoC tests

Mobile micro-electrophoretic device

HemeChip

Ryan Ung et al. Blood 2015;126:3379
Sickle cell detection using a smartphone

S. M. Knowlton¹, I. Sencan¹, Y. Aytar¹, J. Khoory¹, M. M. Heeney¹, I. C. Ghiran* & S. Tasoglu*¹

difference in magnetic levitation between an AA-RBC and an SS-RBC
A rapid paper-based test for quantifying sickle hemoglobin in blood samples from patients with sickle cell disease


- no specific equipment
- result in less than 35 minutes
- by a trained but unqualified personnel
- quantitative?
- cost lower than 0,2 €

decreased HbS solubility
DNA-based PoC tests?

The Q-POC™ diagnostic platform will play a key role in supporting the control, management and eradication of global diseases.

Prof Sir John Burn
Interests of PoC tests in Africa

• **Cost:** affordability
• **Accessibility:** at the patient’s bed; remote areas
• **Reduced delay of diagnosis:** lower risk of loosing affected babies
• **Large indications:**
  – Newborn screening (maternities) and/or early diagnosis (during vaccine schedules)
  – Before any first blood transfusion
  – Identification of carriers among pregnant women to be followed by selective screening
  – Premarital test
New developments in SCD testing

- Point of Care (PoC) tests
- High-throughput newborn screening platforms
Conventional methods

Isoelectric focusing (IEF)
- manual
- lower cost

- HPLC
- Capillary electrophoresis
  - semi-automated
  - highest cost (kits)

Confirmatory tests
- IEF
- HPLC
- Capillary electrophoresis

70-80,000 tests/year

Innovative high throughput methods

Mass spectrometry (MALDI-TOF)
- fully automated
- high cost of material
- no reagents

2-300,000 tests/year
**Conventional methods**

Isoelectric focusing (IEF)
Capillary electrophoresis

**Hbs** separated according to charge

HPLC

**Hbs** separated according to charge/polarity

70-80,000 tests/year

**Innovative high throughput methods**

Mass spectrometry (MALDI-TOF)

**Globin chains** separated according to mass

2-300,000 tests/year

**Newborn Screening**

**Confirmatory tests**
- IEF
- HPLC
- Capillary electrophoresis
MALDI separates $\beta^S$ from other $\beta$-globin chains

mass spectrum

$\beta^S$ chain 15837

15867 $\beta^A$ chain

as a first-line screen, MALDI identifies the $\beta^S$ chain
SS and S$\beta^\circ$ sickle syndromes are identified directly
automatic spectrum analysis software is easily implemented

as for the other screening strategies a 2nd line method is necessary
to distinguish FAS carriers from other compound heterozygotes
SC, SD$^{\text{Punjab}}$, SO$^{\text{Arab}}$ ...

healthy FA
carrier FAS
or compound heterozygote
patient FS

2 platform configurations can be envisaged

- **low throughput screening**
  - low level of automation
  - 100 à 400 samples/day
  - instruments shared with other applications (bacterial typing)
  - preparation of samples manually
  - target: low-income countries

- **high throughput screening**
  - high level of automation
  - 1000-2000 samples/day
  - 100% automation
  - low cost (<2 €)
  - target: countries with national NBS program

Patrick Ducoroy
MALDI-MS distinguishes AS / SS / Sβ+

Ratios of peaks intensity $\alpha$: $\beta$: $\gamma$ combined with TIC (total number of ions) allow separate:
- AS and SS and Sβ+
Two groups of Sβ-thalassemia patients: Sβ+ and Sβ°.

Ratios of peaks intensity α: β: βs: γ combined with TIC (total number of ions) allows to detect:
- Sβ° and Sβ+ (and to distinguish SS and Sβ°)
Detection of β-thalassemia by MALDI-MS

Patrick Ducoroy
The burden of the hemoglobinopathies

• The years lived with disability (YLDs) for hemoglobinopathies and SCD is 10,197, a dramatic observation since the YLDs for cardiovascular disorders is 21,985

• The disability-adjusted life years (DALYs) to measure the disease burden for hemoglobinopathies and SCD is 15,640, an impressive figure compared to the DALYs for diabetes that is 75,000


Courtesy of Lucia De Franceschi
The challenge:
Increase life-expectancy via early diagnosis and prevention of severe complications

Daily penicillin: 1986
Newborn screening: 1988
Immunization

Hydroxycarbamide (HU): 1995
Trans-Cranial Doppler: 1998
Rational transfusion therapy
MERCI

THANK YOU

TERIMA KASIH

Laboratory of Excellence on the Red Cell