EBH- Tutorial
Sickle cell anemia
Sara T O Saad
• A 20 yo afro-descendent man, from Bahia state, was referred to the outpatient clinic of UNICAMP with hypothesis of sickle cell anemia. Clinical exam showed moderate jaundice and pallor and a leg ulcer in the right side. Precordial systolic murmur was detected in the heart examination. BP= 110 x 70 mmHg

• He referred hepatitis diagnosis at 12 yo. In the last year, he had been admitted to the Emergency Room, for 3 days, due to abdominal pain. His parents and all 7 sisters and brothers were healthy

• He had been working since 15 yo, as a barman, cook assistant or door-keeper
Laboratory exams

- Hb 8.7 g/dL, RBC 2.45 x 10^{12}/L, MCV=99fl, MCH= 36.7pg.  reticulocytes- 14,0% WBC= 11.6 x 10^9/L, Platelets= 580 x 10^9/L

Blood smear showed numerous sickle RBC, RBC with Howel Jolly bodies, poikylocytosis.

- Electrophoresis of Hb= HbS + HbA2 + Hb F
  Hb A2= 2.2%; HbF = 10.5%
  - Haplotype = BEN/BEN
  - alpha thal-negative

- LDH= 1500U/L
### Adverse effects prediction

Miller et al, NEJM 2000

<table>
<thead>
<tr>
<th>Decrease</th>
<th>Increase</th>
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<tbody>
<tr>
<td>↓ Hemoglobin</td>
<td>↑ Hemoglobin</td>
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<tr>
<td>↓ Hb F</td>
<td>↑ Leukocytes</td>
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<tr>
<td>↑ Painful crisis</td>
<td>Alpha-thal (+)</td>
</tr>
<tr>
<td>Alpha-thal (-)</td>
<td>Acute anemia</td>
</tr>
<tr>
<td>LDH</td>
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<table>
<thead>
<tr>
<th>Outcomes</th>
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<tbody>
<tr>
<td>Death, stroke, leg ulcer</td>
</tr>
<tr>
<td>Death, ACS, Osteonecrosis</td>
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</tr>
<tr>
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<tr>
<td>Osteonecrosis</td>
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<td>Stroke</td>
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<tr>
<td>Death, Osteonecrosis</td>
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<tr>
<td>Death, stroke</td>
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<tr>
<td>Pulmonary hypertension, leg ulcer, priapism, stroke</td>
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</tbody>
</table>
Haplotypes in Brazilian populations

- **São Paulo**: 45% CAR/Ben, 11% Ben/Ben; 34% CAR/CAR,

- **Bahia**: 46%-55% CAR/Ben, 21%-20% Ben/Ben, 21%-16% CAR/CAR, 11%-9% other

- **Pernambuco**: high frequency of CAR. Low frequency of tBenin.

- **Pará**: 66% CAR, 22% Benin, 11% Senegal, 1% Cameroon

- **Ben/CAR predominates in São Paulo and Bahia**
### Hematologic Values and Fetal Hemoglobin: Interaction of α-Thalassemia-2 with βs-Gene-Cluster Haplotypes

<table>
<thead>
<tr>
<th></th>
<th>HbF (%)</th>
<th>α α / α α</th>
<th>α -/ α α</th>
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<tr>
<td></td>
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</tr>
<tr>
<td>CAR/CAR</td>
<td>4.9 ± 2.9</td>
<td>2.8 ± 2.3</td>
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<tr>
<td>CAR/Benin</td>
<td>7.3 ± 4.7*</td>
<td>6.5 ± 3.9</td>
<td></td>
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<tr>
<td>Benin/Benin</td>
<td>8.3 ± 3.0*</td>
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<table>
<thead>
<tr>
<th></th>
<th>HbF (g/dl)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>α α / α α</td>
<td>α -/ α α</td>
</tr>
<tr>
<td>CAR/CAR</td>
<td>0.37 ± 0.22</td>
<td>0.32 ± 0.28</td>
<td></td>
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<tr>
<td>CAR/Benin</td>
<td>0.60 ± 0.47*</td>
<td>0.53 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>Benin/Benin</td>
<td>0.64 ± 0.21*</td>
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</table>

*P < 0.05"
Laboratory exams

• Unconjugated bilirubin = 6.34 mg% (n < 0.8)
  Conjugated bilirubin = 1.12 mg% (n < 0.4)
• Hepatitis B, C and HIV- negative serology

• Chest XR- cardiomegaly
• Abdominal US- atrophy of spleen, hepatomegaly and cholelithiasis
Laboratory exams

- Creatinine clearance = 219 ml/min
- Serum sodium = 134 mEq/L
- Serum potassium = 4.6 mEq/L

- Urine
  - Density = 1010
  - pH = 5.5
  - Protein - +
  - Urobilinogen - +
  - Bilirubin +
  - Hemoglobin +
Kidney lesions

- Early and severe sickling in kidney
- Begin in the first year and continue for all life
Renal medullary

Hypoxia, hypertonicity, acidosis

\textbf{vasa recta obliteration}

Hyposthenuria

Ischemy of kidney papilla and medullary

Infarction and fibrosis
Glomeruli

Pathogenesis is not completely known

- Sickle RBC phagocytosis by mesangial cells
- Nephritis by immune complex (autoantigens discharged during ischemy)
- Glomerular lesion due to hyperfiltration

Glomerular hypertrophy

- Pathological findings similar to arterial hypertension
- Good Response to ACEi
Follow up 1983-1988

- 2mg of Folic acid/day was prescribed

- In 1985, a second leg ulcer, in the left side, appeared. For leg ulcer many treatments were used as follows:
  - Cleanliness and Unna boot
  - Neomicin for local infection
  - Skin Graft
  - Rest and RBC transfusion

  The leg ulcer was resolved in 4 years (1987)
17% SS patients

- 12 y.o.
- Related to anemia
  - Hypoxia
  - Hyperdynamic flow
  - Hemolysis- ↓NO
Follow up 1990

• Patient referred frequently abdominal pain with colics and vomiting after meals.
  – Ultrasonography
    • Cholelithiasis
    • Enlargement of the liver

• Cholecistectomy was indicated

• Before surgery
  – Acute cholecystitis - treated with antibiotics.
  – Thrombosis in the left arm - treated with heparin and oral anticoagulant
Cholelithiasis and choledocholithiasis

- Due to increased excretion of bilirubin
  - More frequent in homozygotes SS
  - Increase prevalence with age

- Cholecystectomy prevents symptoms
- Surgery after biliary crisis, but not in the acute episode.
- Gilbert Syndrome is a risk factor

Laboratory and clinical data of patients with sickle cell anemia subdivided by their UDP-glucuronosyltransferase 1 (UGT1A) genotype

<table>
<thead>
<tr>
<th>UGT1A genotype</th>
<th>Mean serum total bilirubin levels (mg/dl)</th>
<th>Mean serum unconjugated bilirubin levels (mg/dl)</th>
<th>Frequency of cholelithiasis (%)</th>
<th>Frequency of cholecystectomy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA₆/TA₆</td>
<td>3.33 ± 0.87</td>
<td>2.42 ± 1.62</td>
<td>58.8 (10/17)</td>
<td>5.5 (1/18)</td>
</tr>
<tr>
<td>TA₆/TA₇</td>
<td>3.95 ± 1.07</td>
<td>3.12 ± 0.94</td>
<td>70.0 (14/20)</td>
<td>36.8 (7/19)</td>
</tr>
<tr>
<td>TA₇/TA₇</td>
<td>6.88 ± 1.52</td>
<td>6.03 ± 1.49</td>
<td>80.0 (8/10)</td>
<td>10.0 (1/10)</td>
</tr>
</tbody>
</table>
Follow up 1990

- Surgery was cancelled
- RBC transfusion was indicated. Patient developed severe reduction of hemoglobin
  - Hb= 5.6g/dL; MCV= 100fl; MCH = 31.7 pg; WBC= 12x 10^9/L, Retcs 800 x 10^9/L, Platelets= 1006 x 10^9/L
- **UGT1A1** promoter polymorphism (Gilbert S.)- Homozygote
- DAT+, Elution test +, auto-Ac Ig-G w/o specificity
- 2 months after - DAT negative
- Serum: anti-C and anti-e (IgG), auto anti-I (IgM)
Follow up-1996

- Moderate increase of left ventricle and atrium dimensions
- Mild insufficiency of mitral and tricuspid valves
- Tricuspid regurgitation < 2.5m/s
- Pulmonary arterial pressure=25 mmHg
- Ferritin = 156 ng/mL
- Leg ulcer in the left side after trauma
- LDH= 1427 U/L
- Refused Hydrea due to leg ulcer
Cardiac complications

• Hypertrophy of left chambers due to anemia

• Hypertrophy of right chambers due to pulmonary hypertension
Follow up 1998

- US= chronic liver disease
- Unconjugated bilirubin 11.9mg/dL (n<0.8)
- Conjugated bilirubin 1.6md/dL (n<0.4)
- AST= 73 U/L (n<37), ALT= 15 U/L (n<40)
- Alkaline phosphatase 301 U/L (n<306)
- GGT 139U/L (n<32)
- BUN=24 mg/dL
- Serum creatinine= 0.48 mg/dL
- Glomerular rate filtration= 126mL/min/1.73m2
- Uric acid= 6.5 mg/dL (N< 7mg/dL)
Follow up – 2004

- Cholecystectomy and liver biopsy performed
- RBC transfusion – 3U – pre-surgery

Liver – normal lobular architecture, congestion, severe chronic cholecistopathy

9 days after transfusion patient developed a severe hemolytic anemia and hemoglobinuria. Hb = 3.9g/dL - retics = 60 x 10⁹/L

- Post – transfusional hyperhemolytic syndrome

DAT +, serum: anti Jkb and anti C
ALT- 43 (n<37); AST= 24 (n<40)
Unconjugated bilirubin= 1.7mg/dL (n<0.8)
Conjugated bilirubin= 2.3 mg/dL (n<0.2)
LDH= 3087 U/L
Follow up – 2004

- Chest pain, fever
- X-R- bilateral pleural effusion –hemorrhagic
- Echocardiography- cardiomegaly, mitral insufficiency, pulmonar hypertension (PAP= 47 mmHg). EF= 74%
- CT high resolution=multiple lung infarctions, obstruction of microcirculation
- Prescribed Hydrea . Discharged 6 months after due to leg ulcer
Follow up – 2006

- Bone densitometry: osteoporosis
- Hb=6.2g/dL, WBC= 6.2 x 10^9/L, Plt= 308 x 10^9/L
- Ret= 343 x 10^9/L
- K= 5.7mEq/L, Na= 138 mEq/L
- Creat= 0.56mg/dL, Urea= 18 mg/dL
- Clear EDTA^{CR} = 114mL/min
Bone densitometry in 65 brazilian adult patients with SCD (20 -64y.o)
Baldanzi et al, 2011

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Normal</td>
<td>12</td>
<td>18.5%</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>37</td>
<td>57.0%</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>16</td>
<td>24.5%</td>
</tr>
</tbody>
</table>
## Correlations

<table>
<thead>
<tr>
<th>BMD X Retics</th>
<th>BMD X GFR</th>
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</table>
| **Osteopenia** $p = \text{n.s}$  
**Osteoporosis** $p < 0.001$ | **Osteopenia** $p = 0.08$  
**Osteoporosis** $p = 0.02$ |
| **BMD X LDH** | |
| **Osteopenia** $p = 0.04$  
**Osteoporosis** $p = 0.005$ | |
Adewoye et al. 2008

**Vitamina D and calcium**

- Treatment of 14 patients with Vit D and calcium for one year
- Improvement in densitometry

**Vit D deficiency:**
- Afro-descendents need 5 to 10 x more sun exposition than whites for production of same amount of Vit D
Follow up – 2006

- Echocardiography = diastolic left ventricle dilation, pulmonar hypertension, mitral and tricuspid insufficiency
- Col = 92mg/dL, Triglicerides = 93 mg/dL
- HDL = 37 mg/dL, LDL = 19 mg/dL
- Microalbuminuria = 87mg/g creatinine
- Prescribed ACEi + Hydrea + EPO
Albuminuria in SCD
Early predictor of glomerular damage

5mg/d Enalapril and albuminuria in SCD

**Enalapril effect on renal function in SCD**

**TABLE**

Clinical and Laboratory Data (Mean ± SD) for Patients With Sickle Cell Anemia Before and During Enalapril Treatment and 2 Years After Discontinuation of the Drug (Follow-Up)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 10)</th>
<th>Sickle Cell Anemia Patients (n = 8)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After 6 Months</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>86.9 ± 8.6</td>
<td>70.4 ± 5.5 †</td>
</tr>
<tr>
<td>Ks (mEq/L)</td>
<td>4.5 ± 0.2</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>FEK (%)</td>
<td>4.2 ± 1.1</td>
<td>2.1 ± 1.0 †</td>
</tr>
<tr>
<td>FELi (%)</td>
<td>28.8 ± 9.1</td>
<td>10.9 ± 6.16 †</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>0.9 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>CreatCl (mL/min)</td>
<td>109 ± 19</td>
<td>178 ± 58 †</td>
</tr>
</tbody>
</table>

†Significantly lower (P = 0.004) when compared to before or follow-up values.

‡P = 0.002 vs. controls.

§P = 0.014 vs. controls.

$P = 0.001 vs. controls.

Significantly different when compared to controls.

MAP = mean arterial pressure; Ks = serum potassium; FEK = fractional excretion of potassium; FELi = fractional excretion of lithium; FENa = fractional excretion of sodium CreatCl = creatinine clearance.

iACE in Cardiac remodelling

- ACEi successfully decreased cardiac remodelling in patients with cardiac dysfunction after acute myocardial infarction.

**Enalapril Therapy in Cardiac Remodelling of Sickle Cell Disease Patients** Carmen S. P. Lima 1,2, Osvaldo M. Ueti 2, Adriana A. Ueti 2, Kleber G. Franchini 2, Fernando F. Costa 1,2, Sara T. O. Saad. *Acta Cardiologica* 2008 Oct;63(5):599-602
Follow up – 2007

- Hb = 5.8-6.4g/dL; MCV > 110fl, Rets 170-220x10^9/L.
- Clear EDTA\textsuperscript{CR} = 101mL/min
- LDH = 1691 U/L
- Microalbuminuria = 226mg/g creatinine
- Increase ACEi dose
- Hydrea + EPO + Calcium + Vit D3
Follow up – 2008

- Microalb = 19 mg/g
- Ferritin = 321 ng/mL Transferrin sat = 55%
- Hb = 8.1 g/dl, WBC = 7.29 x 10^9/l (2.71 gran)
- Plt = 396 x 10^9/L, ret = 250 x 10^9/L, HbF = 23.3%
- LDH = 884 U/L
- Creatinine = 0.67 mg/dL, U = 29 mg/dL
- Leg ulcer on the left side – Topical autologous Platelet Rich Plasma – complete cicatrization
- Hydrea, EPO, ACE
Follow up 2011- EPO + HU + ACE

- 48 y.o – PA = 110X60 mmHg

Hb= 8.56 g/dl, Neutrophils-5 x10^9/L,
Platelets 483 x 10^9/L,
HbF= 15.3%
LDH= 1174U/L
Microalb= 37.84 mg/g
Creatinine= 0.53mg/dL, U= 15mg/d
- Hydrea –
Maximal tolerated dose
prevention of organ damage

- Neutrophils ~ 2 x $10^9$/L
- Platelets > 100 x $10^9$/L
- Reticulocytes > 50 x $10^9$/L
Preservation of spleen and brain function in children with sickle cell anemia treated with hydroxyurea.
Hankins JS, Helton KJ, McCarville MB, Li CS, Wang WC, Ware RE.

PATIENTS AND METHODS:
Retrospective study

RESULTS:
43 children had spleen function measured both at baseline and on therapy. After a median of 2.6 years (range, 0.2-8.6 years) of hydroxyurea at maximum tolerated dose (MTD), six patients (14%) completely recovered splenic function and two (5%) had preserved splenic function. These eight children had a greater hemoglobin (Hb) concentration on hydroxyurea therapy than those without splenic function (9.1 vs. 8.6 gm/dl, P = 0.01). Of 25 children with brain MRI/MRA studies performed before initiating hydroxyurea and on therapy, 24 (96%) had no change in brain ischemic lesions compared with pre-treatment studies, after a median of 2.9 years of treatment.
Conclusion

• Even patients with non-severe SCA may have severe complications
• Intervention: early Hydrea, Bone Marrow Transplantation?
• Hepatobiliary complications may be severe in Gilbert Syndrome homozygotes
  Intervention : early surgery
Conclusions

• Prevention of nutritional deficiency of Calcium and Vit. D (infancy and adolescence)
• iACE for kidney and heart function improvement
• EPO + Hydrea increase Hb levels and may substitute RBC transfusions
• Topical Autologous Platelet Rich Plasma for leg ulcer treatment