Blood Group Genotyping in Germany

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Clinical management of D antigen in Germany

- two anti-D monoclonals
  - that do not bind DVI
  - German guidelines since 1996
- RhIg
  - prenatal & post partum
- CcEe antigens, if
  - girl
  - woman < 45 years
  - multiple transfusions
  - immunhematologic problems
- D antigen most immunogenic
  - because RhD protein is completely lacking in D neg.
  - most important blood group system encoded by proteins

Transfus Clin Biol 13(2006)4
Clinical applications

- Patients with weak D phenotypes
  - **prevalent weak D should be transfused D pos.**
- Maternal care
- Blood donors
- Anti D and family planning
Distribution of molecular weak D types in Europeans

- weak D type 1 to 4: 94%
- less frequent weak D types combined: 6%
- often confirmed in Europeans
- details vary among European populations
- e.g. Portuguese: weak D type 2 most frequent

- Blood 93(1999)385
weak D types among transfusion recipients with anti-D

- 31 observations since 1998
- prevalent weak D: auto-anti-D only n=24
- weak D type 4.0 n=3, low titer
- allo-anti-D * n=4 weak D type 4.2, 11 & 15
Clinical management of patients with a weak D phenotype

<table>
<thead>
<tr>
<th>Weak D phenotype</th>
<th>Prevalence in Germany</th>
<th>Haploype association</th>
<th>Transfusion recipients with anti-D (n)*</th>
<th>Recommended management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>0.2964%</td>
<td>CDe</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Type 2</td>
<td>0.0759%</td>
<td>cDE</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Type 3</td>
<td>0.0219%</td>
<td>CDe</td>
<td>0</td>
<td>n.a.</td>
</tr>
<tr>
<td>Type 4.0</td>
<td>0.014%</td>
<td>cDe</td>
<td>3</td>
<td>See remark</td>
</tr>
<tr>
<td>Type 4.1</td>
<td>0.023%</td>
<td>cDe</td>
<td>0</td>
<td>n.a.</td>
</tr>
<tr>
<td>Type 4.2</td>
<td>Rare</td>
<td>cDe</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Type 5</td>
<td>0.0035%</td>
<td>cDE</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Type 11</td>
<td>&gt;0.0009%, 0.0009%</td>
<td>CDe, cDe</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Type 15</td>
<td>Rare</td>
<td>cDE</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Type 19</td>
<td>0.067%</td>
<td>CDe</td>
<td>0</td>
<td>n.a.</td>
</tr>
<tr>
<td>Type 20</td>
<td>0.024%</td>
<td>cDE</td>
<td>0</td>
<td>n.a.</td>
</tr>
<tr>
<td>Other types</td>
<td>Rare - Variable</td>
<td></td>
<td>0</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

- Flegel WA: Curr Opin Hematol 2006 in press
Patient testing for clinically relevant weak D types

- may save 3% – 5% of all D neg. units for D neg. recipients who really need them
- one test per patient
- CE-labelled test kits
- offered as a service in Germany since 2000

- protocol details published in e.g.
  - Flegel WA
  Curr Opin Hematol 2006 in press
Clinical applications

- Patients with weak D phenotypes
- Maternal care
  - prenatal diagnostics
  - RhIg in pregnancy
- Blood donors
- Anti D and family planning
Prenatal diagnostics

• blood group genotyping is standard care
  • puncture of fetal cord contra-indicated, if done for blood group typing only
    • German consensus statement
      Infusionsther Transfusionsmed 27(2000)215

• typical diagnostic requests in Germany
  • from amniotic fluid or its cell culture
    • in order of frequency:
      • D
      • Rhesus phenotype (CcEe)
      • K
      • Fy, Jk etc. (sporadic requests only)

• fetal \textit{RHD} from maternal plasma
  • not widely available in Germany at this time
RhIg during pregnancy may be dropped, ...

- ... if mother carries certain weak D types
- ... if fetus types D neg. from maternal plasma

- Blood 93(1999)385
- Curr Opin Hematol 2006 in press
- Obstet Gynecol 106(2005)841
Health and cost benefits
Rhlg may be dropped, ...

• ... if mother carries certain weak D types
  • may save 3% – 5% of all anti-D shots
  • one test per mother’s lifetime
  • test pays for itself
    • health benefit for mother may come for free

• ... if fetus types D neg. from maternal plasma
  • may save 40% of prenatal anti-D shots
  • one or more tests per pregnancy
  • test may pay for itself
Regulatory issues
RhIg may be dropped, ...

- ... if mother carries certain weak D types
- CE-labelled test kits
- compatible with current guidelines
- offered as a service in Germany since 2001
- ... if fetus types D neg. from maternal plasma
- no test kit available
- guidelines would need to be relaxed
- currently not offered as a service in Germany
Clinical applications

• Patients with weak D phenotypes
• Maternal care
• Blood donors
  • screen for DEL, weak D and D⁺/D⁻ chimera among serologically D neg. donors
• Anti D and family planning
Rationale:
to improve RBC unit safety

- potential immunogenicity of weak D, DEL and D⁺/D⁻ chimera
- *RHD* with very weak D antigen expression
  - detectable by adsorption/elution or flow cytometry
  - it should be remembered: there are *RHD* alleles without any serologically detectable D antigen
- Transfusion 45(2005)1547
Routine testing 1/02 – 12/05 at our blood service

- testing of all first time donors for D antigen
  - serology according to German guidelines
  - oligoclonal anti D plus antiglobulin test in gel technique
- screening of all serologically D negative first time donors for *RHD* gene
  - 29,823 in 4 years
- PCR screening of pools of 20 donors
  - PCR-SSP for *RHD* intron 4
- all novel *RHD* alleles characterized
  - PCR, sequencing, testing for DEL
## Distribution among Rhesus phenotypes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>N</th>
<th>RHD positive</th>
<th>DEL positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccddee</td>
<td>27,859</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Ccddee</td>
<td>1,241</td>
<td>44</td>
<td>23</td>
</tr>
<tr>
<td>ccddEe</td>
<td>679</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>CCddee</td>
<td>20</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CcddEe</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ccddEE</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CCddEe</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>29,823</td>
<td>61</td>
<td>26</td>
</tr>
</tbody>
</table>
**RHD alleles expressing DEL differ among ethnic groups**

<table>
<thead>
<tr>
<th>Population</th>
<th>Any RHD allele</th>
<th>DEL phenotype</th>
<th>Prevalent RHD alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europeans</td>
<td>0.2%</td>
<td>1 in 1000</td>
<td>RHD(IVS3+1G&gt;A), RHD-CE(8-9)-D, RHDΨ, weak D type 11 in CDe*</td>
</tr>
<tr>
<td>Africans</td>
<td>10%</td>
<td>&lt;1 in 100</td>
<td>RHDΨ, Cde s</td>
</tr>
<tr>
<td>East Asians</td>
<td>30%</td>
<td>1 in 3</td>
<td>RHD(K409K), weak D type 15, weak D type 17</td>
</tr>
</tbody>
</table>

RHD genotyping in donors: improves RBC unit safety

- by moving donors with
  - weak D, DEL & D+/D- chimeric RBC populations
- to the D positive donor pool
- obviates need for very sensitive anti-D test and its quality assurance (cost savings)
- adaptation to allele distributions in different populations required
  - protocols for e.g. East Asia
    Transfusion 45(2005)345 and in press
Clinical applications

- Patients with weak D phenotypes
- Maternal care
- Blood donors
- Anti D and family planning
  - genotype father for \( RHD \) heterozygosity, if mother carries anti D
Detection of *RHD* heterozygous status in fathers

- **hybrid Rhesus box**
  - Problem
    - *Rhesus box* variants
  - Blood 95(2000)3665

- **RHD/RHCE dosage**
  - Problem
    - *RHCE-D-CE* alleles, like *D--*
  - Transfusion 46(2006)1343

Fig. 3. Analysis of ccDec samples with large D antigen density by quantitative PCR.
PCR-SSP for *RHD* deletion

- detection of *RHD* heterozygosity in a father
**RHD genotyping**

- **DD** homozygous
- **Dd** heterozygous
- **dd**
Regulatory and liability issues

*RHD* heterozygous status

- hybrid *Rhesus box*
- PCR with sequence specific primers (PCR-SSP)
- Problem
  - *Rhesus box* variants primarily in Africans
- Resolution
  - *RHD/RHCE* dosage by quantitative PCR

歌声 would be both tests, but one test is better than none

- CE-labelled test kits
- offered as a service in Germany since 2001
- Blood 95(2000)3665
- Transfusion 45(2005)327 & 338

- *RHD/RHCE* dosage
- quantitative real time PCR
- Problem
  - *RHCE-D-CE* alleles, like *D--primarily in Europeans (?)*
- Resolution
  - hybrid *Rhesus box* by PCR-SSP

- no test kit available
- offered as a service in Germany (not in Ulm)
- Transfusion 46(2006)1343
Summary
Clinical applications in Germany

- Patients with weak D phenotypes
  - prevalent weak D should be transfused D pos.
- Maternal care
  - prenatal diagnostics
  - RhIg in pregnancy
- Blood donors
  - screen for DEL, weak D and D⁺/D⁻ chimera among serologically D neg. donors
- Anti D and family planning
  - genotype father for RHD heterozygosity, if mother carries anti D
- Next step forward
  - mass scale genotyping, e.g. biochips
Supplementary material
Editorials 2005

- Transfusion 45 (Mar 2005) 293
  characterized and thus detected. Recently in Germany, PCR-based quality control of 15,045 serologically defined D− units revealed that 39 of them carried RHD alleles.¹⁰

- Transfusion 45 (Apr 2005) 466
  many, RHD genotyping of D− first-time donors (among 500,000 donations/year) has been a routine procedure

- Transfusion 45 (Oct 2005) 1547
  Since 2000, Flegel⁵ has tested all his D− donors in Ulm, Germany, by a molecular method to ensure detection of all forms of RHD.
Anti D immunization by “D neg.” blood donors

- weak D type 2
  - 1 immunization
    - Transfusion 40(2000)428

- D+-/- chimera
  - > 2 immunizations
    - BMC Genet 2(2001)10

- weak D type 26
  - 1 immunization in pregnancy
    - Transfusion 45(2005)527
    - Editorial in Transfusion 45(2005)466

- DEL phenotype
  - $RHD$(IVS5-38del4)
  - 1 immunization
    - Transfusion 45(2005)520

- $RHD$(K409K)
  - most prevalent DEL type worldwide
  - 1 secondary immunization
    - Transfusion 45(2005)1581
Superior sensitivity: mistyped by routine serology

- missed among 8,442 “D neg.” donors
  - 3 partial D
  - 1 weak D type 2
  - 1 D+/- chimera
- D+/- chimera → total of 13 donations
  - caused anti-D in the latest 2 eligible transfusions
- it may be wise to revisit older serologic results

- BMC Genet 2(2001)10
Frequency distribution

- RHD(IVS3+1G>A)
- RHD-CE(8-9)-D
- RHDΨ
- RHD(M295I)
- RHcE(1-3)-D(4-10)
- RHD(K409K)
- RHD(W16X)
- RHD(147delA)
- RHD(343delC)
- RHD(IVS3+2T>A)
- RHD(93-94insT)
- RHD(L153P)
- RHD(785delA)
- RHD(1253-53insT)
- RHD(V56M; W90X)
- RHD(712delG)
- RHD(660delG)
- RHD(R318X)
- RHD(Y269X)

Orange bars indicate novel alleles among serologically
D neg. or DEL
Novel alleles per year
RH blood group system
RhD and RhCE proteins

- RhD vs. RhCE (yellow)
  - C/c (green)
  - E/e (black)
- weak D
  - (red/orange)
- partial D
  - (blue/light blue)
Type of D variant correlates with type of molecular variant

<table>
<thead>
<tr>
<th>Type of variant</th>
<th>Type of variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>D antigen</td>
<td>Allele (molecular type)</td>
</tr>
<tr>
<td>D category</td>
<td><strong>RHD-CE-D</strong> hybrid alleles</td>
</tr>
<tr>
<td>partial D: European</td>
<td>exofacial single missense</td>
</tr>
<tr>
<td>partial D: African</td>
<td>exofacial dispersed missense</td>
</tr>
<tr>
<td>weak D</td>
<td>non exofacial single missense</td>
</tr>
<tr>
<td>DEL</td>
<td>e. g. splice site mutation</td>
</tr>
<tr>
<td>D negative</td>
<td><strong>RHD</strong> deletion</td>
</tr>
<tr>
<td></td>
<td>non functional rearranged</td>
</tr>
<tr>
<td></td>
<td>non functional nonsense</td>
</tr>
</tbody>
</table>