Cut-off value of the Bethesda assay and detection of low titre inhibitors in previously untreated children with severe haemophilia A

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on behalf of the PedNet and RODIN Study Group

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Background

About one third of patients with severe haemophilia A (sHA) develop inhibitors towards infused FVIII.

The inhibitor risk depends on genetic and non genetic factors

After the introduction of recombinant FVIII products the reported inhibitor incidences have increased. It is still unclear whether this is caused by higher intrinsic immunogenicity, by more frequent testing, higher awareness in reporting low titre inhibitors or higher sensitivity of the performed tests with a lower cut-off.

Aim

To describe the impact of the cut-off value of the Bethesda assay on the overall inhibitor outcome in previously untreated (PUPs) children with sHA.

Patients & Methods

The RODIN Study (Research of Determinants of Inhibitor development) included PUPs with sHA for the first 75 exposure days (E.D)

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Uniform prospective data and well-defined outcomes were collected from this patient cohort regularly tested for inhibitors in 29 haemophilia centres in Europe, Israel and Canada. The investigators are members of the European Paediatric Network for Haemophilia Management (PedNet) and/or the RODIN Study group.

The centres, which all used the Nijmegen modification of the Bethesda assay, were asked to report on the cut-off value that was used during the study period (2000-2010) and also the frequency of testing during the first 20 E.D and between 20 -75 E.D.

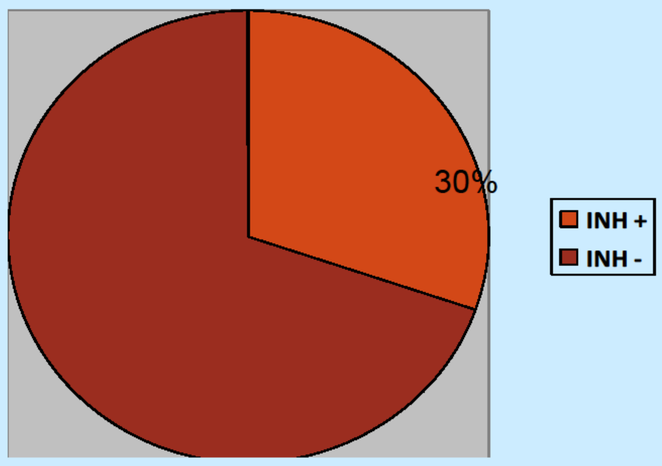
Outcomes

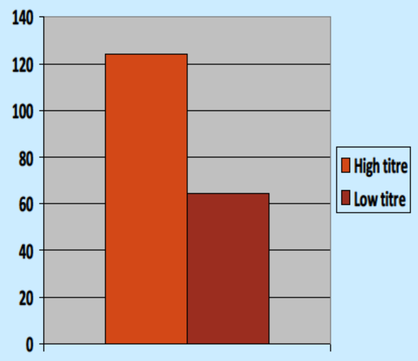
The primary outcome of the RODIN Study was clinically relevant inhibitor development
(defined as at least two positive inhibitor titres combined with a decreased in vivo FVIII recovery up to the 75th exposure day).
A positive inhibitor titre was defined according to the cut-off level of the inhibitor assay used in each center's laboratory.
The FVIII recovery was considered to be decreased, when it was less than 66% of the expected FVIII activity level 15 minutes after infusion of FVIII.

The secondary outcome was high titer inhibitor development
(defined as the occurrence of a clinically relevant inhibitor with a peak titer of at least 5 BU/mL)

Results

In total **621** patients were eligible, whereof 188 (30%) developed an inhibitor





Of these N=188 inhibitor patients N=124 (66%) had a high titre and N=64 (34%) had a low titre inhibitor

Patients developed inhibitors after a median of 15 E.D (inter-quartile range (IQR) 10-20 days), at a median age of 15.5 months (IQR 10.7-19.6 months)

There were 10 centres that used low cut-off values (0.3 and 0.4 BU/ml) and 19 using high cut-off values (0.5 and 0.6 BU/ml, the latter being the highest cut-off value for positivity).

Influence of cut-off level on the detection of the number and titre of inhibitors

	Cut off <=0.4 BU/ml	Cut off >0.4<=0.6 BU/ml
Number of centers	10	19
N Total number of patients	203	418
N Total number of Inhibitor patients	56 (27,5%)	132 (31.6 %)
N Number of high titre patients	37 (66%)	87 (66%)
N Number of low titre patients	19 (34%)	45 (34%)

The number of PUPs tested in the 10 centers with the lower cut-off value were N= 203 (32.7%) and with the higher cut-off value N= 418 (67.3%)

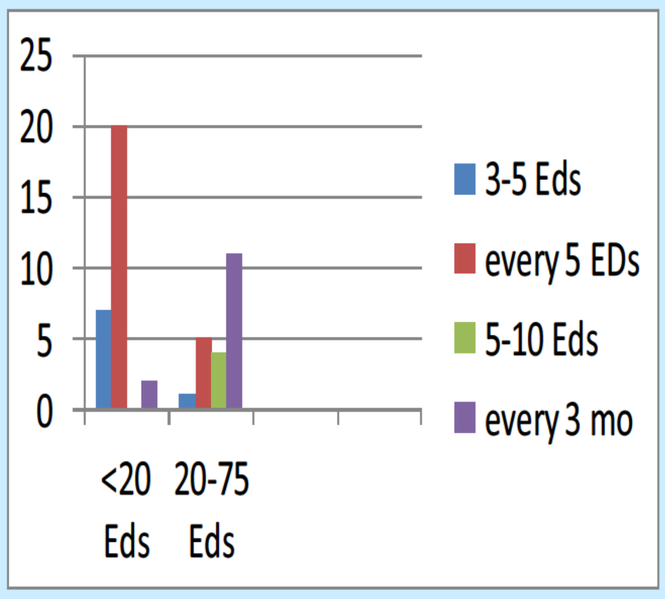
From the center's with the lowest cut-off, 27.5% of the patients developed an inhibitor

For the centers with a cut-off value of < 0.6 BU, 31.6% (132/418) of them developed an inhibitor

The proportion of high and low titre patients was similar

Frequency of testing for inhibitors from 0-20 and 20-75 exposure days

	0-20 EDs	20-75 EDs
Every 3-5 exposure days	7	1
Every 5 exposure days	20	5
Every 5-10 exposures		4
Every 3 months	2	11
Other	None	6 *?



* Other was a broad variation of 3-5 EDs and every 1-2 months.

Conclusions

A lower cut-off value did not influence the detection rate of inhibitors

Also the frequency of low versus high titre inhibitor patients was similar for both cut-off values

Since all centers tested frequently no effect of testing on the results was expected

The PedNet and RODIN Study Centres

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