Establishment of anti-Hepatitis C Virus IgG Avidity Test for Dried Serum/Plasma Spots

Amare Eshetu[1], Andrea Hauser[1], Matthias an der Heiden[2], Daniel Schmidt[2], Karolin Meixenberger[1], Stefan Ross[3], Martin Obermeier[4], Robert Ehret[4], Claus-Thomas Bock[5], Barbara Bartmeyer[2], Viviane Bremer[2], Norbert Bannert[1]

[1]HIV and Other Retroviruses, Robert Koch Institute, Berlin, Germany, [2]HIV/AIDS, STI and Blood-borne Infections, Robert Koch Institute, Berlin, Germany
[3]Universität Duisburg-Essen, Essen, Germany, [4]Medizinisches Infektiologiezentrum Berlin, Berlin, Germany, [5]Division for Viral Gastroenteritis and Hepatitis Pathogens and Enteroviruses, Robert Koch Institute, Berlin, Germany,

Introduction & Objective

Hepatitis C virus (HCV)-antibody detection from dried serum or plasma spots (DS/PS) is established for surveillance purposes. An additional information on the duration of HCV infection is required to improve surveillance and to target or evaluate public health interventions. However, an acute infection is often asymptomatic and it is difficult to discriminate a recent infection from a long standing infection that is positive for HCV RNA using traditional methods. This however, can be achieved by measuring the binding capacity (or avidity) of antibodies (AB) to the HCV antigens because the avidity of ABs increases over time in the presence of the antigen. This approach to measure the recency of infection can be principally used for both plasma/serum and dried blood spot samples. We report here the establishment of an in-house AB-avidity based HCV recency assay for use of DS/PS that takes different HCV genotypes into account in order to distinguish best between recent and long-term infections.

Materials and Methods

HCV immunoglobulin G (IgG) antibody avidity assay was developed by using a Monolisa Anti-HCV test (BioRad) and a modified protocol from Patel *et al* (2016)*. The assay was used to calculate i) avidity index (AI), a ratio of optical density values from a two-well assay, where one well is treated with Diethylamine to dissociate low-avidity antibodies and a second untreated well is used as a control ii) mean duration of recent infection (MDRI), the average time an individual is identified as having been recently infected ii) false-recent rate (FRR), the proportion of DS/PS collected >104 weeks after HCV seroconversion and misclassified as 'recent'. iii) false long-term infection rate (FLTR), the proportion of samples collected <26 weeks after HCV seroconversion misclassified as `longstanding´. Three different sample panels were used for validation: 1) to demonstrate the increase in HCV IgG antibody avidity over time, to calculate the FLTR and MDRI 2) to calculate the FRR in chronic infections 3) to analyze assay performance of resolved infections (figure 1).

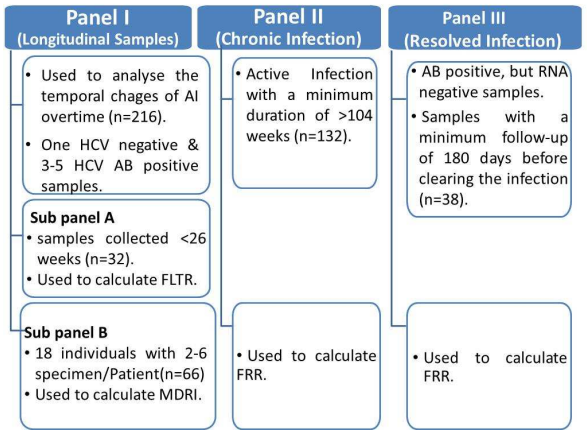

Figure 1: HCV-seroconversion panels used for validation of IgG antibody avidity assay.

Results

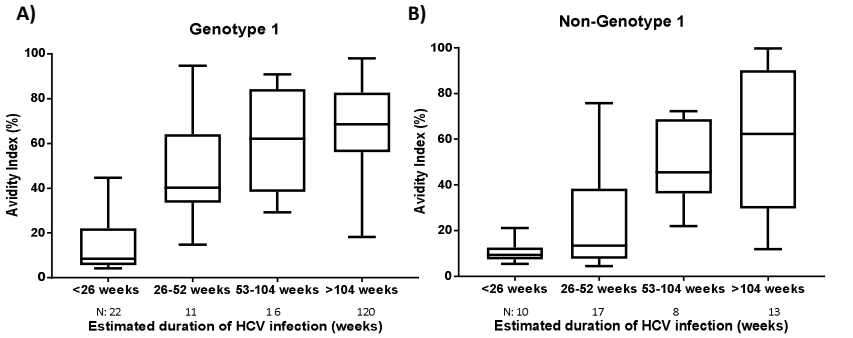

Figure 2: Differential increase in HCV IgG antibody avidity over time for genotype 1 (A) and non-genotype 1(B) The median AI was shown to increase progressively with duration of infection category for both genotype 1 and non-genotype 1 infections. Higher AI values were generally obtained for genotype 1 samples.

Table 1: Sensitivity, Specificity, and Mean Duration of Recent Infection according to selected cut-off avidity index (AI) values calculated for the longitudinal dataset (n=66).

AI cut-off (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)	MDRI (days)	(95% CI)
20	72.4	72.9	72.6	233	(149 , 449)
25	78.3	79.3	78.8	264	(179, 634)
30	81.5	82.1	81.8	268	(183, 695)
35	82.2	80.9	81.5	325	(228, 445)
40	85.7	88.2	86.9	364	(223, 485)
45	80.3	70	75.1	517	(16, 643)

The optimal AI of 40% resulted in an MDRI of 364 days (95%CI: 223, 485) for all subtypes with a specificity and sensitivity of 88.2% and 85.7%, respectively.

Table 2: Estimated `false recent rate´ (FRR) and `false long term rate´ calculated from the `long term´ (> 104 weeks) and `recent´ infection panel (< 26 weeks) according to AI cut-off and genotype.

	Avidity Index (%)					
	20	25	30	35	40	45
FRR (%)						
All genotypes (n=132)	2.2 (0.5-6.5)	6.8 (3.2-12.5)	9.1 (4.8-15.3)	11.4 (6.5-18)	13.6 (8.3-20.7)	16.7 (10.7-24.1)
Genotype 1 (n=119)	0.8 (0-4.6)	5.8 (2.4-11.7)	7.5 (3.5-13.9)	9.2 (4.7-15.9)	11.7 (6.6-19)	14.3 (8.5-21.9)
Non-genotype1 (n=13)	15.4 (1.9-45.4)	15.4 (1.9-45.4)	23.1 (5-53.8)	30.6 (9.1-61.4)	30.6 (9.1-61.4)	38.5 (13.9-68.4)
FLTR (%)						
All genotypes(n=32)	21.8 (9.3-40)	9.3 (2-25)	6.3 (0.8-20.8)	3.1 (0.1-16.2)	3.1 (0.1-16.2)	0 (-)
Genotype 1 (n=22)	27.2 (10.7-50.2)	13.6 (2.9-34.9)	9.1 (1.1-29.2)	4.5 (0.1-22.8)	4.5 (0.1-22.8)	0 (-)
Non-genotype1 (n=10)	10 (0.3-44.5)	10 (0.3-44.5)	10 (0.3-44.5)	10 (0.3-44.5)	10 (0.3-44.5)	0 (-)

The FRR for the optimal AI cut off 40% calculated from samples with a long term infection was 13.6% for all genotypes, 11.7% for genotype 1, and 30.6% for non-genotype 1 HCV infections. However, at the same AI cut-off value, the FLTR was 3.1% for all genotypes, 4.5% for genotype 1, and 10% for non-genotype 1 HCV infections.

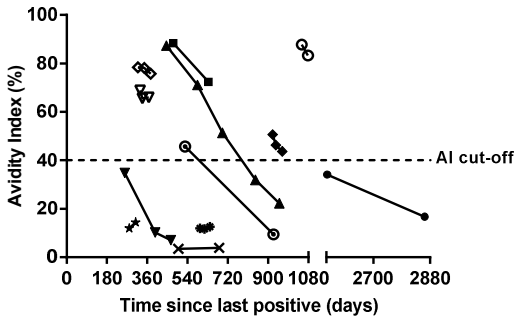

Figure 3: Changes in AI values over time for patients with resolved HCV infection.

All the samples with a resolved infection with a minimum follow-up of 180 days before clearing the infection have been shown to have a decline of the AI overtime. Furthermore, the FRR at optimal AI cut-off 0f 40% was 50% (19/38; 95%CI: 16.2-59.8).

Conclusion

For all genotypes, stronger antibody avidity is associated with the days post infection. The newly established assay performs well for most of the prevalent genotypes in Germany. However, a larger sample size is required to obtain MDRI and to confirm FRR and FLTR for non-genotype 1 infections. The decline in the avidity index and the high FRR amongst resolved infections indicate that this group could be mistaken for recent infection and that viral load will be a valuable supplementary marker to identify recent infections.

*Patel *et al* (2016). Use of Hepatitis C Virus (HCV) Immunoglobulin G Antibody Avidity as a Biomarker to Estimate the Population-Level Incidence of HCV Infection. *J. Infect. Dis.*

Acknowledgement
The study is funded by the German Federal Ministry of Health.

Amare Eshetu
Phone : +49(0)30 18754 2819
AlemayehuA@rki.de