HCV core antigen is an alternative marker to HCV RNA for evaluating active HCV infection: implications for improved diagnostic option in an era of affordable DAAs

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The core antigen of the hepatitis C virus (HCV Ag) presents an alternative marker to HCV RNA when screening patients for HCV viremia. This study sought to evaluate the utility of HCV Ag as a marker to assess active HCV infection in individuals residing in an HCV endemic area. From 298 HCV-seropositive individuals evaluated for the presence of anti-HCV antibody, HCV Ag and HCV RNA, anti-HCV antibody was detected in 252 individuals (signal-to-cutoff ratios ≥ 5), HCV RNA was detected in 222 individuals (88%), and HCV Ag was reactive (≥ 3 fmol/L) in 220 individuals (87%). HCV genotype 1, 3, and 6 were identified. HCV Ag significantly correlated with HCV RNA irrespective of HCV genotype and/or HBV co-infection (log HCV RNA = 2.67 + 0.95 [log HCV Ag], R^2 = 0.890, $p < 0.001$). To predict HCV viremia (HCV Ag ≥ 3 fmol/L), the accuracy, sensitivity, specificity, positive predictive value, and negative predictive value were 99%, 99%, 100%, 100% and 97%, respectively. We concluded that HCV Ag was a good surrogate marker for HCV RNA and could be used to diagnose active HCV infection in a resource-limited setting. As a result, a cost-effective strategy for screening and identifying active HCV carriers using HCV Ag detection would enable more patients access to efficacious and increasingly affordable direct-acting antivirals (DAAs) for the treatment of HCV infection.

1   **HCV Core Antigen is an Alternative Marker to HCV RNA for Evaluating**

2   **Active HCV Infection: Implications for Improved Diagnostic Option in an**

3   **Era of Affordable DAAs**

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14 **ABSTRACT**

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16 when screening patients for HCV viremia. This study sought to evaluate the utility of HCV Ag

17 as a marker to assess active HCV infection in individuals residing in an HCV endemic area.

18 From 298 HCV-seropositive individuals evaluated for the presence of anti-HCV antibody, HCV

19 Ag and HCV RNA, anti-HCV antibody was detected in 252 individuals (signal-to-cutoff ratios $\geq$

20 5), HCV RNA was detected in 222 individuals (88%), and HCV Ag was reactive ($\geq$ 3 fmol/L) in

21 220 individuals (87%). HCV genotype 1, 3, and 6 were identified. HCV Ag significantly

22 correlated with HCV RNA irrespective of HCV genotype and/or HBV co-infection (log HCV

23 RNA = 2.67 + 0.95 [log HCV Ag], R^2 = 0.890, $p < 0.001$). To predict HCV viremia (HCV Ag $\geq$

24 3 fmol/L), the accuracy, sensitivity, specificity, positive predictive value, and negative predictive

25 value were 99%, 99%, 100%, 100% and 97%, respectively. We concluded that HCV Ag was a

26 good surrogate marker for HCV RNA and could be used to diagnose active HCV infection in a

27 resource-limited setting. As a result, a cost-effective strategy for screening and identifying active

28 HCV carriers using HCV Ag detection would enable more patients access to efficacious and

29 increasingly affordable direct-acting antivirals (DAAs) for the treatment of HCV infection.

INTRODUCTION

31 Infection with hepatitis C virus (HCV) can lead to acute or chronic hepatitis, liver fibrosis,

32 cirrhosis, end-stage liver disease, and hepatocellular carcinoma *(Perz et al., 2006)*. Disease

33 progression after HCV infection depends on factors including gender, coinfection with HIV,

34 alcohol consumption, and duration of chronic infection *(Hajarizadeh et al., 2013 ; El-Serag,*

35 *2012*). The global population seroprevalence of anti-HCV antibodies is estimated at around

36 1.6%, or roughly 115 million people *(Gower et al., 2014)*. The presence of HCV antibodies can

37 be found in spontaneous clearance, resolved infection post-treatment or persistently active

38 disease. Early treatment for HCV infection especially in the acute phase can facilitate viral

39 clearance and prevent chronic infection, thereby limiting HCV-induced liver damage and

40 mortality *(Jaeckel et al., 2001; Gerlach et al., 2003; Singal et al., 2010)*.

41 Screening for anti-HCV antibody status often facilitates HCV surveillance in the

42 community *(Meffre et al., 2010; Garcia Comas et al., 2015; Morisco et al., 2016)*. Although

43 simple, such assay cannot differentiate between past and present infection and requires

44 supplemental HCV RNA testing to confirm active HCV infection and monitor antiviral

45 treatment. Despite its high sensitivity and reliability, HCV RNA assay involving nucleic acid

46 testing (NAT) and quantitative real-time RT-PCR requires skilled laboratory personnel,

47 sophisticated equipment, and expensive reagents *(Roth et al., 2012; Halfon et al., 2006)*.

48 Therefore routine screening using these tests is limited particularly for many resource-

49 constrained setting. In contrast, testing for HCV core antigen (HCV Ag) presents a more

50 attractive alternative owing to the lower cost and short turnaround time. HCV Ag has been

51 shown to be an indirect marker for HCV replication comparable to the detection of HCV RNA

52 *(Schuttler et al., 2004; Bouvier-Alias et al., 2002; Ottiger et al., 2013; Florea et al., 2014)*. In

53   addition to serving as a reliable marker to diagnose active HCV infection, HCV Ag can also be

54   used to evaluate the treatment response to antiviral therapy *(Mederacke et al., 2009; Rockstroh et*

55   *al., 2017; Alonso et al., 2017).* Another advantage of HCV Ag assay is that it can often be

56   performed on the same instrument and simultaneously with the anti-HCV assay, an added value

57   when determining the HCV prevalence in the community *(Kuo et al., 2012; Mixson-Hayden et*

58   *al., 2015).*

59   New and effective therapeutic direct-acting antivirals (DAAs) taken orally have become

60   widely available in recent years. DAAs have advanced HCV therapy with higher rates of

61   sustained-virological response (SVR) post-treatment than those obtained from traditional

62   interferon-based therapies independent of HCV genotype (*Sulkowski et al., 2014; Zeuzem et al.,*

63   *2014; Gane et al., 2015).* Despite the availability of these highly efficient drugs, asymptomatic

64   nature of HCV infection and expensive diagnostic screening process represent major obstacles in

65   identifying and treating HCV-infected individuals *(Cox, 2015).* Therefore, a simple, cost-

66   effective testing feasible for routine HCV screening would be ideal for a developing country

67   such as Thailand. In this study, we aimed to evaluate the diagnostic utility of HCV Ag as an

68   alternative to HCV RNA to identify active HCV infection in a relatively high endemic area. We

69   also assessed the cost feasibility and savings of implementing such program towards public

70   health policy in an era of DAAs.

71   **MATERIALS AND METHODS**

72   This follow-up study examined the prevalence and genotypes of HCV found in Petchabun and

73   Khon Kaen province and comprised part of a previous HCV surveillance project involving 3077

74   samples from high- and average-level HCV endemic areas *(Wasitthankasem et al., 2017)*. All

75   HCV-seropositive individuals were informed of their status and invited to participate in

76   confirmatory testing for HCV infection using anti-HCV, HCV Ag, and HCV RNA assays

77   combined with liver enzyme levels and liver stiffness tests in March 2016. The study protocol

78   was approved by the institutional review board of the Faculty of Medicine, Chulalongkorn

79   University (IRB No. 258/58). Objectives of the study were explained to the patients and written

80   informed consents were obtained.

81   **Study subjects**

82   Of the 310 eligible individuals with positive anti-HCV Ab results, 270 subjects participated in

83   the follow-up study (Fig. 1). Another 28 subjects with anti-HCV positive status established after

84   blood donor screening were also enrolled in this study. In all, 298 subjects (aged 34–64 years)

85   provided demographic information and blood samples. Liver function data including aspartate

86   aminotransferase (AST) and alanine transaminase (ALT) levels were obtained, and HCV viral

87   loads were determined from blood plasma. The degree of liver fibrosis was assessed by transient-

88   electrography (FibroScan, Echosens, Paris, France). Hepatitis B virus (HBV) and HIV status

89   were previously determined using HBsAg and HIV Ag/Ab automated screening (ARCHITECT,

90   Abbott Diagnostics, Wiesbaden, Germany).

91   **Anti-HCV serological test**

92   All sera were tested for an anti-HCV antibody using automated chemiluminescent microparticle

93   immunoassays (CMIA) (ARCHITECT anti-HCV assay, Abbott Diagnostics, Wiesbaden,

94   Germany). Signal-to-cutoff ratio (S/CO) indicated the presence of anti-HCV, with S/CO ≥ 1.00

95   automatically assigned as reactive by the ARCHITECT i1000SR platform. The U.S. Centers for

96   Disease Control and Prevention (CDC) recommended the true predictive result above 95% when

97   the S/CO ratio is ≥ 5. Therefore, the latter was adopted, and reactive anti-HCV samples were

98   further categorized based on this criterion.

99   **HCV RNA measurement**

100   Plasma HCV RNA level was determined using an automated *in vitro* reverse-transcription

101   polymerase chain reaction (RT-PCR) assay (Abbott RealTime HCV assay, Abbott Molecular,

102   IL). The lower and upper limits of quantitation of this kit were 12 and 100,000,000 IU/mL, or

103   log 1.08 and log 8.00 IU/mL, with a linearity range between 8.21 log IU/mL to 0.91 log IU/mL

104   (according to the manufacturer's instructions). Samples over the upper detection limit were

105   examined for viral genotype and were not included in further analyses. Samples with HCV RNA

106   level < 12 IU/mL and ≥ 12 IU/mL were defined as negative and positive for HCV RNA,

107   respectively.

108   **HCV Ag measurement**

109   HCV core antigen (HCV Ag) quantitation was determined by automated assays using an

110   Architect i2000SR system (ARCHITECT HCV Ag, Abbott Diagnostics, Wiesbaden, Germany).

111   This assay is a CMIA based on the interaction between monoclonal anti-HCV and HCV Ag. A

112   HCV Ag concentration < 3 fmol/L was interpreted as nonreactive, and the other samples with

113   higher titers were interpreted as being reactive for HCV Ag. The detection limit ranged from 0 to

114 20,000 fmol/L. Therefore, samples with HCV Ag over the limit of detection (20,000 fmol/L)

115 were further diluted (by a factor 1:9), and re-examined by automated dilution protocol in the

116 Architect i2000SR. The machine automatically calculated and reported the final HCV Ag

117 concentration.

118 **HCV genotyping**

119 Genotype was determined based on the nucleotide sequence of the HCV core region. HCV RNA

120 was extracted, and nested RT-PCR of the partial core region was performed on the samples with

121 HCV RNA $\geq$ 12 IU/mL. Primer pairs of 954F/410 R were used in the first round, and 953F/951R

122 were used in the second round, as previously described *(Wasitthankasem et al., 2015)*. Target

123 PCR amplicon of the core region was purified (ExpinGel SV, GeneAll Biotechnology, Seoul,

124 Korea) and sequenced.

125 **Data and statistical analysis**

126 Continuous variables such as the level of AST and ALT were described in particular groups with

127 < 30 U/mL and $\geq$ 30 U/mL, whereas liver stiffness was categorized according to the Metavir

128 score in which F0-F2 equals liver stiffness < 9.5 kPa and F3-F4 equals liver stiffness $\geq$ 9.5 kPa

129 *(Castera et al., 2008)*. The difference between groups and group means was evaluated by Chi-

130 squared test and one-way ANOVA using a Bonferroni model, respectively. Association between

131 the level of HCV RNA and HCV Ag was analyzed in log scale by a linear regression model.

132 Differences of regression data among genotypes were analyzed by general linear model and

133 univariate analysis. The regression model evaluated the odds ratios and 95% confidence intervals

134 of demographic information associated with HCV RNA status. A p-value < 0.05 was considered

135 statistically significant. Sensitivity, specificity, positive predictive value (PPV), negative

136   predictive value (NPV), and accuracy of the diagnostic tests for anti-HCV and HCV Ag markers

137   were calculated using HCV RNA status as a gold standard. All statistical analyses were

138   performed using SPSS version 11.5for Windows (SPSS, Chicago, IL).

139   **RESULTS**

140   **Demographic information of participants**

141   The study design is shown in Figure 1. From 298 individuals in this study, 290 were reactive to

142   anti-HCV (S/CO ≥ 1.0). There were 242 (83.4%) males and 48 (16.6%) females (mean age 50.4

143   ± 7.2 and 48.4 ± 8.4 years, respectively). Many of the anti-HCV reactive individuals had

144   elevated levels (≥30 U/mL) of AST (65.5%), ALT (51.7%) and advanced liver fibrosis (47.1%,

145   Metavir score: F3-F4) (Table 1). Among the anti-HCV reactive individuals, 13 of 266 with

146   available HBV status were found reactive to HBsAg, while 1 was reactive to HIV Ag/Ab.

147   Additional measurements in HCV antibody-positive samples showed the presence

148   (222/290) and absence (68/290) of HCV RNA (Fig. 1). One sample with HCV RNA exceeding

149   the upper limit of detection (more than log 8 IU/mL) was considered RNA positive but not

150   included in further analyses. All but two of the HCV RNA-positive subjects were reactive to

151   HCV Ag (≥3 fmol/L). A majority of samples with positive viral RNA showed elevated liver

152   enzymes (AST = 77.0% and ALT = 62.2%) and had severe fibrosis (54.3%) (Table 1). There

153   were significant associations between positive HCV RNA and male gender (OR 2.3, 95% CI:

154   1.2, 4.5, $p = 0.013$), elevated liver enzyme levels (AST; OR 8.6, 95% CI: 4.7, 16.0, $p < 0.001$

155   and ALT; OR 7.7, 95% CI: 3.9, 15.1, $p < 0.001$) and advance liver stiffness (OR 3.9, 95% CI:

156   2.1, 7.1, $p < 0.001$). Genotyping of HCV revealed 61 samples with genotype 1 (subtype 1a = 44

157   and 1b = 17), 71 samples with genotype 3 (subtype 3a = 69 and 3b = 2) and 90 samples with

158   genotype 6 (subtype 6c = 1, 6f = 72, 6i = 4 and 6n = 13).

159   **Anti-HCV, HCV Ag and HCV RNA status**

160   A schematic diagram of anti-HCV, HCV Ag, and HCV RNA testing in this study is shown in

161   Figure 2. Thirty-eight out of 290 individuals with anti-HCV S/CO < 5.0 and HCV Ag < 3.0

162   fmol/L tested negative for HCV RNA. Of the 252 samples with anti-HCV S/CO ≥ 5, 220

163   samples with HCV Ag ≥ 3 fmol/L were positive for HCV RNA. In 32 samples with anti-HCV

164   S/CO ≥ 5 and HCV Ag < 3 fmol/L, 2 samples were positive for HCV RNA.

165   Among 13 HBV reactive samples, there were 6 HCV RNA positive samples, 5 of which

166   were positive for HCV Ag. One sample showed evidence of triple infection with HIV, HBV, and

167   HCV.

168   **Correlation of anti-HCV and HCV Ag with HCV RNA level**

169   Examination of the correlations among anti-HCV, HCV Ag, and HCV RNA levels in log scale

170   showed that anti-HCV significantly correlated with HCV RNA (log HCV RNA = -0.882 + 5.492

171   [log anti-HCV], $R^2 = 0.534$, $p < 0.001$) (Fig. 3a). Using an anti-HCV S/CO cut-off at 5, there

172   were 30 false-positive (FP) samples with HCV RNA < 12 IU/mL (Table S1) and no false-

173   negatives (FN). Better correlation was observed between HCV Ag and HCV RNA level (log

174   HCV RNA = 2.67 + 0.95 [log HCV Ag], $R^2 = 0.890$, $p < 0.001$) (Fig. 3b). Taking an HCV Ag

175   cut-off at 3 fmol/L, only two FN with HCV RNA levels of 501 and 1445 IU/mL were identified

176   (Fig. 3b and Table S1). Both samples had HCV Ag levels of 0.0 and 2.1 fmol/L and were of

177   genotype 3a (Fig. 4b).

178   The mean levels of HCV RNA in the samples with genotype 1 (log 6.0 ± 0.9 IU/mL),

179   genotype 3 (log 5.6 ± 1.1 IU/mL), and genotype 6 (log 6.1 ± 0.8 IU/mL) were determined along

180   with the mean HCV Ag (genotype 1, log 3.5 ±1.0 fmol/L; genotype 3, 2.9 ±1.1 fmol/L; genotype

181   6, 3.8 ±0.7 fmol/L) (Table 2). Genotypes showed significant differences in the log mean of both

182   HCV RNA and HCV Ag ($p = 0.001$ and $p < 0.001$, respectively). Samples with HCV genotype 3

183   had lower levels of HCV RNA and HCV Ag than genotype 1 ($p = 0.014$ and $p = 0.001$,

184   respectively) and 6 ($p = 0.001$ and $p < 0.001$, respectively). Based on the regression data, HCV

185   Ag showed greatest correlation with HCV RNA in samples with genotype 1 (log HCV RNA =

186   $2.97 + 0.87$ [log HCV Ag], $R^2 = 0.905$, $p < 0.001$) followed by genotype 6 (log HCV RNA =

187   $2.323 + 1.01$ [log HCV Ag], $R^2 = 0.870$, $p < 0.001$), and genotype 3 (log HCV RNA = 2.75 +

188   0.97 [log HCV Ag], $R^2 = 0.848$, $p < 0.001$) (Fig. 4 and Table 2). Finally, the slight difference in

189   regression data among genotypes was not statistically significant ($p = 0.102$).

190   There was no significant difference in the log mean of HCV RNA and HCV Ag between

191   HCV mono-infection (N = 215, log 5.9 ± 1.0 IU/mL and log 3.4 ± 1.0 fmol/L) and HCV/HBV

192   co-infection (N = 5, log 5.7 ± 1.7 IU/mL and log 3.0 ± 1.5 fmol/L) (p-value > 0.05). HCV Ag

193   highly correlated with HCV RNA in HCV/HBV co-infection (log HCV RNA = 2.44 + 1.08 [log

194   HCV Ag], $R^2 = 0.968$, $p = 0.002$), while correlation of these two markers between mono-

195   infection and co-infection was not significantly different ($p = 0.119$). The one sample with triple

196   infection (HCV/HBV/HIV) had viral load and HCV Ag at log 6.7 IU/mL and log 4.10 fmol/L,

197   respectively.

198   **Evaluation of anti-HCV and HCV Ag assay in predicting HCV infection**

199   To identify HCV viremia, an anti-HCV S/CO cut-off at 5 showed 89.7% accuracy, 100%

200   sensitivity, and 55.9% specificity. Positive predictive value (PPV) and negative predictive value

201   (NPV) were 88.1% and 100%, respectively (Table 3). By applying the HCV Ag assay to predict

202   active HCV infection, a cut-off of 3 fmol/L had the greatest accuracy (99.3%), with 100%

203   specificity, 99% sensitivity, 100% PPV, and 97% NPV. Using both anti-HCV and HCV Ag

204   markers (S/CO $\geq$ 5 and HCV Ag $\geq$ 3 fmol/L), diagnostic predictions of HCV viremia were

205   similar to those using HCV Ag marker alone (Table S1).

206   **DISCUSSION**

207   Timely HCV treatment especially during the acute phase of infection has shown promise in

208   multiple HCV genotypes and results in improved prognosis *(Jaeckel et al., 2001)*. Identifying

209   actively infected individuals is therefore important in reducing HCV transmission and disease

210   burden, especially in areas of endemicity. Although, anti-HCV screening is the primary test for

211   diagnosis of HCV infection, supplemental testing is still needed to discriminate between resolved

212   and viremic infection. The results of this study suggest that anti-HCV Ab tests had a fair

213   correlation with HCV RNA and are appropriate for primary screening due to its high sensitivity.

214   Previously, it was suggested that an appropriate cut-off could predict HCV viremia *(Kuo et al.,*

215   *2012)*. Thus, this study applied a S/CO cut-off at 5.0 for predicting a true antibody-positive result

216   ≥ 95% recommended by the U.S. CDC (https://www.cdc.gov/hepatitis/hcv/labtesting.htm).

217   Among samples with anti-HCV S/CO ≥ 5, HCV RNA was positive in 88.1% (222 of 252), while

218   those with S/CO < 5.0 were all negative. The latter may have resulted from a false-positive anti-

219   HCV result or an individual with resolved disease and/or low antibody titer. Using this cut-off

220   ratio to predict HCV infection, high diagnostic sensitivity (0.0% FN and 11.9% FP) was noted.

221   When the cut-off ratio was adjusted to 15.0, better predictive values for HCV infection were

222   obtained with excellent PPV (98.8%) and specificity (98.5%), albeit with poor NPV (32.8%) and

223   sensitivity (38.3%) (Table S2). Thus, anti-HCV testing with S/CO at 5.0 was appropriate for the

224   first-line screening.

225   Unlike anti-HCV Ab, HCV Ag showed excellent diagnostic validity and correlation with

226   HCV RNA. At a cutoff of 3 fmol/L, HCV Ag was a good predictive marker of HCV viremia

227   with 0 (0.0%) FP and 2 (2.9%) FN cases. The validity of HCV Ag testing was better than the

228   anti-HCV marker that had 99.1% sensitivity, 100% specificity, 100% PPV, 97.1% NPV and

229   99.3% accuracy. It has been suggested that combining anti-HCV and HCV Ag at the appropriate

230   cutoff point would improve the predictive value for HCV viremia, but the validity was similar to

231   HCV Ag alone when combining those two markers *(Kuo et al., 2012)*. In addition, HCV Ag

232   correlates well with RNA levels and may therefore serve as a predictor for response-guided

233   therapy and for monitoring treatment response (*Nguyen et al, 2017*; *Alonso et al., 2017*).

234   The 2 FN cases from the HCV Ag assay had low viral loads and were of genotype 3.

235   These results may be related to the viral level and detection limit of the respective genotypes.

236   Low levels of HCV RNA tended to increase the coefficient of variation and contributed to FN on

237   HCV Ag assays *(Aghemo et al., 2016; Ottiger et al., 2013)*. Several studies suggest that the

238   lower limit of HCV Ag detection is equivalent to HCV RNA between 1000 and 5000 IU/mL,

239   therefore low RNA level in these 2 samples (501 IU/mL and 1445 IU/mL) may have contributed

240   to the FN result *(Florea et al., 2014; Freiman et al., 2016; Ottiger et al., 2013)*. In addition,

241   adequate analytical sensitivity at 3 fmol/L of HCV Ag of genotype 3 required approximately

242   1002 IU/mL of HCV RNA, which was higher than for genotype 1 (896 IU/mL) *(Ross et al.,*

243   *2010; Tillmann, 2014)*. Similar to this cohort, greater variation in genotype 3 samples with low

244   HCV Ag but high HCV RNA was previously reported *(Ottiger et al., 2013; Mederacke et al.,*

245   *2009)*. Discordant result found in this study (0.7%, 2/290) could potentially be resolved with a

246   confirmatory RNA testing (e.g. reactive anti-HCV and non-reactive HCV Ag) *(Rockstroh et al.,*

247   *2017)*.

248   A meta-analysis demonstrated that HCV Ag had excellent correlation with viremia

249   despite limitations on the effect of HCV genotypes and HBV or HIV co-infection *(Freiman et*

250   *al., 2016)*. A significant difference was observed in the log mean of viral RNA and antigen levels

251   among genotypes, but this was not apparent in the correlation between these two markers.

252   Previous studies also reported that HCV Ag of genotype 1, 2, 3 and 4 had good correlation with

253   viral load *(Mederacke et al., 2009; Ottiger et al. 2013; Ross et al., 2010)*. Our study also showed

254   good correlation between HCV Ag and RNA in HCV/HBV co-infection samples. However, poor

255   correlation in the HBV co-infection group may sometimes occur especially with genotypes other

256   than genotype 1 *(Mederacke et al., 2012)*. This may be attributed to very low or very high

257   concentration of HCV Ag relative to HCV RNA in some samples. Therefore, HCV Ag retesting

258   should be performed in the samples with very low or very high concentrations. Excellent

259   correlation had been reported in HCV/HIV co-infection *(Mederacke et al., 2012; Thong et al.,*

260   *2015)*, but that information was insufficient in triple infection samples. We found only one

261   sample with HCV/HBV/HIV triple infection and elevated HCV viremia and HCV Ag. Further

262   studies are required to elucidate the correlations in subjects with dual infections and triple

263   infections.

264   Previous studies examining HCV Ag in HCV infection were conducted in industrialized

265   countries *(Mederacke et al., 2009; Mixson-Hayden et al., 2015; Rockstroh et al., 2017; Freiman*

266   *et al., 2016)*, while data from low- to middle-income countries (LMIC) have been scarced *(Khan*

267   *et al., 2017; Freiman et al., 2016)*. Our finding provided the evidence of the utility of HCV Ag

268   for HCV screening in LMIC endemic for HCV due to the assay's high diagnostic validity and

269   lower cost than an RNA assay, which potentially could improve access to treatment and care in

270   LMIC *(Wang et al., 2017; Khan et al., 2017)*.

271   Currently, the World Health Organization initiated a goal to eradicate viral hepatitis by

272   increasing the proportion of diagnosed persons by up to 90% by the year 2030 *(WHO, 2017)*.

273   This policy will require a substantial investment. Fortunately, affordable and highly efficacious

274   DAA treatment is increasingly accessible *(Nebehay, 2017)*. To enhance the likelihood of

275   eradicating HCV, results from our study and others suggest an alternative screening and

276   diagnostic strategy to treat HCV infection, especially in the era of DAAs (Fig. 5). When

277   screening initially involves anti-HCV antibody and confirmatory HCV Ag assay, followed by

278   HCV RNA testing on HCV Ag-negative samples, a cost-effective algorithm by us demonstrate a

279   considerable reduction, roughly 48% compared to the standard expenditure (standard algorithm;

280   anti-HCV test followed by RNA assay, File S1) and is consistent with that proposed by others

281   (*Jülicher et al, 2017*). This strategy would effectively provide equal diagnostic performance that

282   supports the possibility of large-scale implementation.

283   In conclusion, our population-based study showed high validity of HCV Ag as a reliable

284   marker for diagnosis of active HCV infection. This marker showed excellent correlation with the

285   viral RNA irrespective of genotypes and HBV co-infection. In addition, HCV Ag can serve as a

286   supplemental marker after anti-HCV testing to reduce the sample number requiring further

287   confirmatory RNA assays. A proposed cost-effective strategy would reduce the financial burden

288   required for national screening and improve access to HCV treatment and care in the era of

289   affordable DAAs.

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Table 1

Demographic data of individuals with anti-HCV positive serology in the cohort. Samples were categorized according to HCV RNA status.

1 **TABLE 1.** Demographic data of individuals with anti-HCV positive serology in the cohort.

2 Samples were categorized according to HCV RNA status.

	HCV RNA -ve (N = 68)	HCV RNA +ve (N = 222)	Total (N = 290)	Odds ratio (95% CI)	*P*-value
Sex (M:F)	50:18	192:30	242:48	2.3 (1.2, 4.5)	0.013
Age (Mean ± SD)	50.4 ± 7.7	50.0 ± 7.3	50.1 ± 7.4	0.9 (0.6, 1.3)	0.687
AST (%)				8.6 (4.7, 16.0)	< 0.001
< 30 U/mL	49 (72.1%)	51 (23.0%)	100 (34.5%)		
≥ 30 U/mL	19 (27.9%)	171 (77.0%)	190 (65.5%)		
ALT				7.7 (3.9, 15.1)	< 0.001
< 30 U/mL	56 (82.4%)	84 (37.8%)	140 (48.3%)		
≥ 30 U/mL	12 (17.6%)	138 (62.2%)	150 (51.7%)		
Liver stiffness[a] (%)				3.9 (2.1, 7.1)	< 0.001
F0-F2	52 (76.5%)	101 (45.9%)	153 (52.9%)		
F3-F4	16 (23.5%)	120 (54.3%)[a]	136 (47.1%)		

4 [a]Liver stiffness (F0-F2, < 9.5 kPa; F3-F4, ≥ 9.5 kPa) could not be determined in 1 individual.

Table 2

Correlation regression between HCV RNA and HCV Ag among different HCV genotypes (in log scale).

1   **TABLE 2.** Correlation regression between HCV RNA and HCV Ag among different HCV

2   genotypes (in log scale).

	Genotype 1 (N = 61)	Genotype 3 (N = 69)	Genotype 6 (N = 90)	TOTAL (N = 220)[a]	*P*-value
HCV RNA log[b] IU/mL (Median)	6.0 ± 0.9 (6.32)	5.6 ± 1.1 (5.89)	6.1 ± 0.8 (6.43)	5.9 ± 1.0 (5.87)	0.001
HCV Ag log[b] fmol/L (Median)	3.5 ± 1.0 (3.79)	2.9 ± 1.1 (3.35)	3.8 ± 0.7 (4.04)	3.4 ± 1.0 (3.7)	< 0.001
Regression data[c]					0.102
R	0.952	0.921	0.933	0.927	
R-square	0.905	0.848	0.870	0.860	
p-value	<0.001	<0.001	<0.001	<0.001	

5   [a]Of 222 detectable HCV RNA samples, one sample with over limit of detection and one sample

6   with undetectable HCV Ag were excluded.

7   [b]HCV RNA and HCV Ag level presented as mean ± SD.

8   [c]Correlation between HCV Ag and HCV RNA in log scale.

Table 3

Diagnostic test evaluation of anti-HCV, HCV Ag and combination of anti-HCV and HCV Ag tests to predict HCV active infection in community screening.

1   **TABLE 3.** Diagnostic test evaluation of anti-HCV, HCV Ag and combination of anti-HCV and

2   HCV Ag tests to predict HCV active infection in community screening.

	% Sensitivity (95% CI)	% Specificity (95% CI)	% Positive predictive value (95% CI)	% Negative predictive value (95% CI)	% Accuracy (95% CI)
Anti-HCV (S/CO = 5.0)	100.0 (98.4-100.0)	55.9 (43.3-67.9)	88.1 (83.4-91.8)	100.0 (90.7-100.0)	89.7 (85.6-92.9)
HCV Ag (3 fmol/L)	99.1 (96.8-99.9)	100.0 (94.7-100.0)	100.0 (98.3-100.0)	97.1 (90.1-99.7)	99.3 (97.5-99.9)
Anti-HCV (S/CO = 5) plus HCV Ag (3 fmol/L)[a]	99.1 (96.8-99.9)	100.0 (94.7-100.0)	100.0 (98.3-100.0)	97.1 (90.1-99.7)	99.3 (97.5-99.9)

4   [a]Anti-HCV (S/CO ≥ 5) plus HCV Ag (≥3 fmol/L) and others.

Figure 1

Figure 1

Evaluation of 298 anti-HCV reactive samples in this study. Anti-HCV reactive samples were collected from individuals who returned to the HCV follow-up study for the treatment program (270 from the previous HCV study and 28 from self anti-HCV screening) (26). Anti-HCV nonreactive samples were excluded, and reactive samples were tested for liver function enzymes, liver stiffness, HCV RNA level and HCV Ag level. Viral genotypes were examined in samples with HCV RNA ≥ 12 IU/mL. AST, aspartase aminotransferase; ALT, alanine transaminase.

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                        ┌─────────────────────────────┐
                        │  310 out of 3077 anti-HCV    │
                        │   reactive samples (26)      │
                        └─────────────────────────────┘
                                      │
                                      ▼
┌──────────────────────┐   ┌─────────────────────────────┐
│ 28 previously known  │ + │ 270 individuals returned for │
│  anti-HCV reactive   │   │  the follow-up study (26)    │
│     individuals      │   └─────────────────────────────┘
└──────────────────────┘                │
                                        ▼
                           ┌─────────────────────────────┐
                           │  298 samples recruited in    │
                           │   the follow-up study        │
                           └─────────────────────────────┘
                                        │
                                        ▼
                           ┌─────────────────────────────┐
                           │     Anti-HCV screening       │
                           └─────────────────────────────┘
```

Anti-HCV nonreactive (S/CO < 1.00)
N = 8 samples [EXCLUDED]

Anti-HCV reactive (S/CO ≥ 1.00)
N = 290 samples

Measurement of liver function enzyme, AST and ALT	Measurement of liver stiffness	Measurement of HCV RNA level	Measurement of HCV Ag level

N = 290 samples

N = 289 samples (one sample with immeasurable stiffness)

HCV RNA < 12 IU/mL; N = 68 samples	HCV RNA ≥ 12 IU/mL; N = 222 samples	HCV Ag < 3 fmol/L; N = 70 samples	HCV Ag ≥ 3 fmol/L; N = 220 samples

HCV genotyping

Figure 2

Figure 2

Schematic diagram of all 290 anti-HCV reactive samples. Anti-HCV reactive samples were categorized based on S/CO ratio = 5. HCV Ag level was determined in all anti-HCV reactive samples and sample with HCV Ag ≥ 3 fmole/L was designated as reactive. HCV RNA level was determined to confirm HCV active infection with the presence of RNA level ≥ 12 IU/mL.

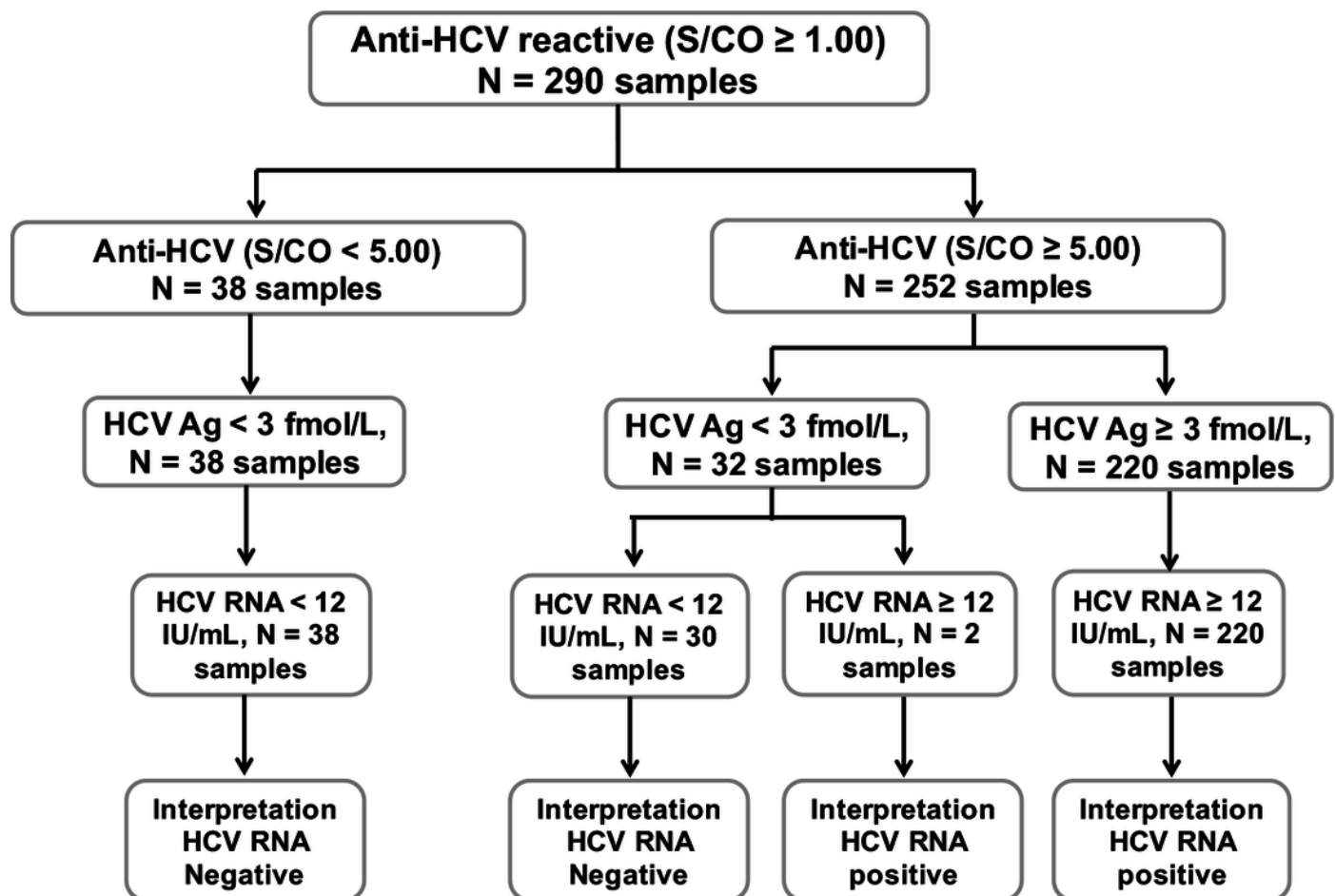
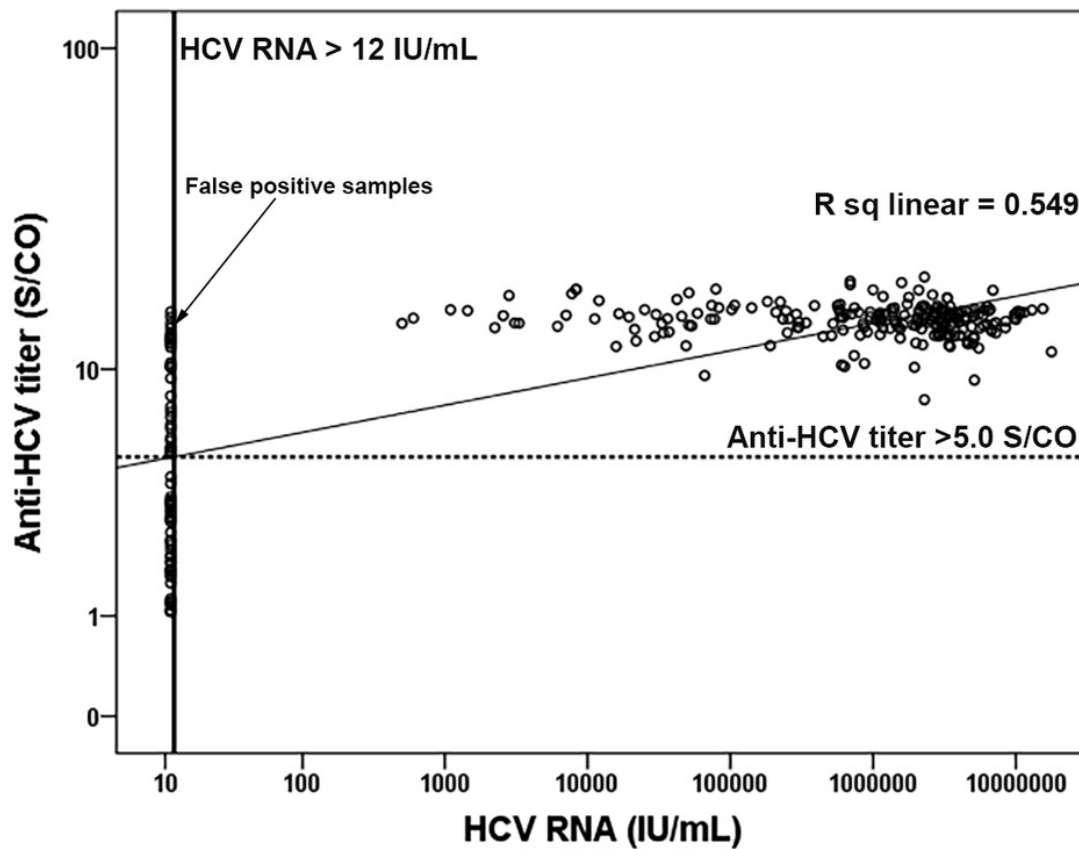



Figure 3

Figure 3

Correlation of anti-HCV and HCV Ag with HCV RNA level. Anti-HCV and HCV RNA level were analyzed in log scale (a). The lower limit of detection line for HCV RNA was 12 IU/mL. The cutoff line of anti-HCV was 5.0 S/CO. HCV Ag and HCV RNA concentration were analyzed in log scale (b). The lower limit of detection line for HCV RNA was 12 IU/mL. The cutoff line of HCV Ag was 3 fmol/L.

a

b

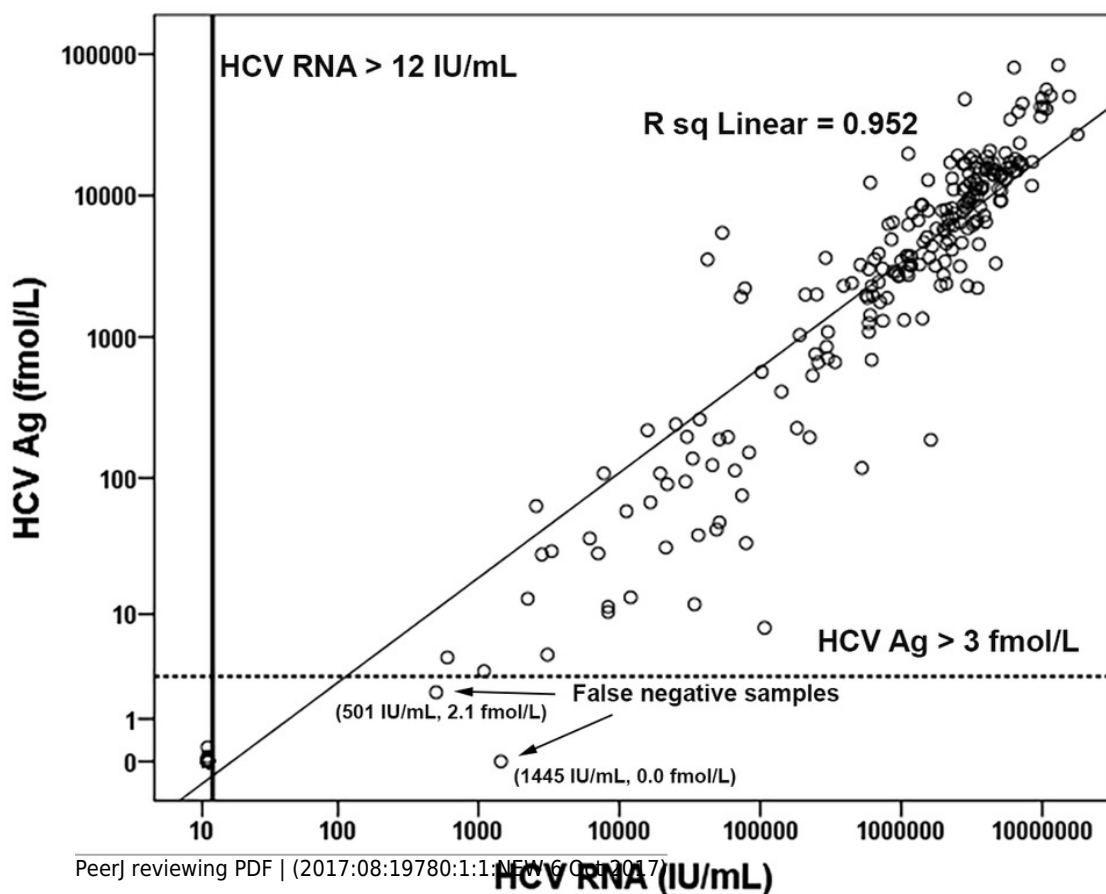



Figure 4

Figure 4

Correlation of HCV Ag with HCV RNA concentration for different genotypes. Comparison of HCV Ag and HCV RNA level was analyzed in log scale for genotype 1 (a), genotype 3 (b) and genotype 6 (c). The lower limit of detection line for HCV RNA was 12 IU/mL. The cutoff line of HCV Ag was 3 fmol/L.

a

b

c

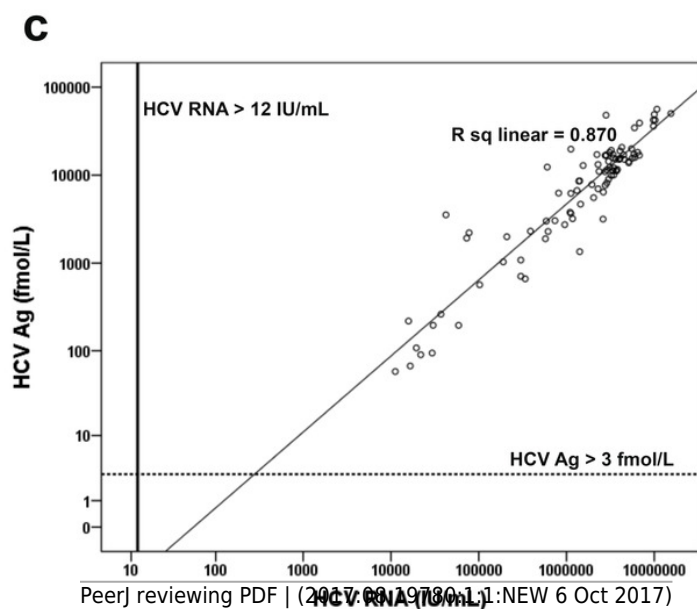
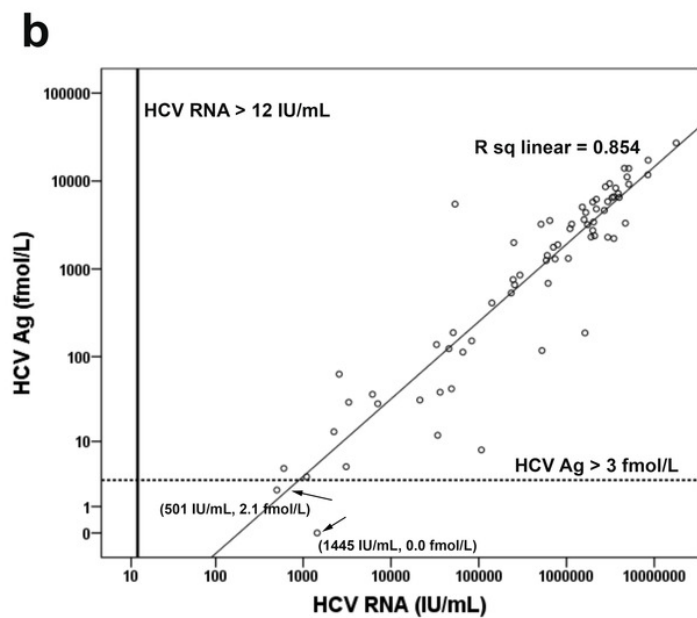
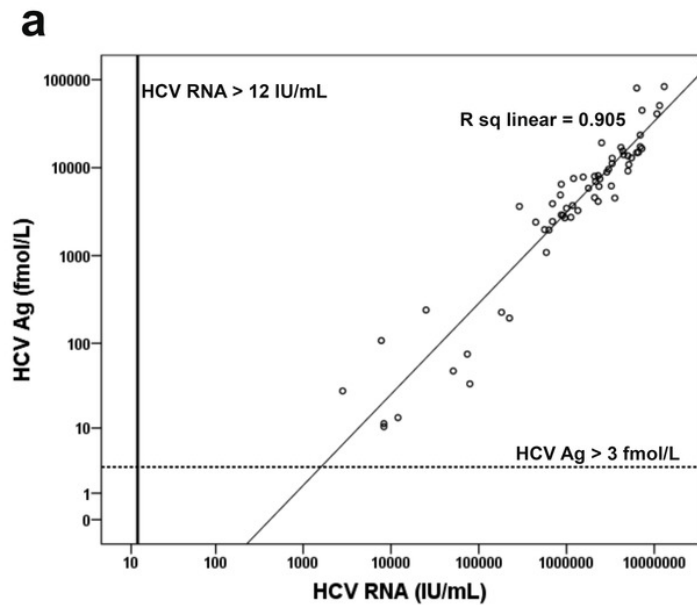



Figure 5

Figure 5

Optional screening strategy for treatment of HCV infection. HCV genotyping is an option depends on IFN- or DAAs-based therapy.