Aberrant Concentrations of Liver-Derived Plasma Albumin mRNA in Liver Pathologies

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BACKGROUND: We hypothesized that liver-derived mRNA, such as ALB (albumin) mRNA, would be released into human plasma with liver cell death.

METHODS: We genotyped ALB mRNA molecules in samples of plasma and whole blood from liver and bone marrow transplant recipients by RNA single-nucleotide polymorphism analysis. Plasma and whole blood ALB mRNA genotypes were compared with the DNA genotypes of the recipients and donors. A reverse-transcription quantitative real-time PCR assay was used to measure plasma ALB mRNA concentrations in 107 patients [hepatocellular carcinoma (HCC), cirrhosis, or chronic hepatitis B (CHB)] and 207 healthy controls.

RESULTS: The RNA genotype data revealed ALB mRNA in plasma to be liver derived, whereas tissue compartments other than the liver also contributed to the ALB mRNA detected in whole blood. Statistically significant increases in plasma ALB mRNA concentrations were observed for HCC, cirrhosis, and active CHB, compared with controls. A cutoff of 835 copies/mL of plasma ALB mRNA identified by ROC curve analysis showed 85.5% diagnostic sensitivity and 92.8% diagnostic specificity for the detection of liver pathologies. Only 21.5% of patients with liver pathologies had increased ALB mRNA concentrations in whole blood. A cutoff of 835 copies/mL of plasma ALB mRNA concentrations, whereas 91.4% had increased plasma ALB mRNA concentrations.

CONCLUSIONS: ALB mRNA is liver specific in plasma, but not in whole blood. Plasma ALB mRNA is increased in some liver pathologies and may be more diagnostically sensitive than α-fetoprotein and ALT.

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The analysis of circulating nucleic acids in plasma offers an avenue for noninvasive monitoring of a variety of physiological and pathologic conditions (1, 2). Numerous applications based on the detection of circulating cell-free nucleic acids in human plasma have been reported for the management of malignancies (3), pregnancy-associated conditions (4), organ transplantation (5), and trauma (6, 7). The fundamental principle underlying these applications relates to the detection in plasma of extracellular nucleic acid molecules derived from diseased organs. Disease-specific genetic signatures that could be exploited from analyzing circulating DNA include the detection of disease-related pathogens (8), disease-specific mutations, and sex and polymorphism differences between a fetus and the mother or a transplant donor and the recipient.

In addition to circulating DNA, analysis of cell-free plasma RNA offers another dimension of opportunity for the development of pathology-related markers (1, 9). Expression profiles unique to an organ or disease could be targeted as the specific nucleic acid signature for detection in plasma. Tumor-derived (10) and placenta-derived (11) RNA species successfully detected in plasma have potential for use in disease assessment (12).

In this study, we explored the possibility of detecting circulating liver-derived mRNA for the assessment of liver pathologies. There is much evidence to suggest that circulating DNA and RNA are released upon cell death (13). Because albumin is the most abundant protein in the body and is synthesized by the liver, we hypothesized that ALB (albumin) mRNA should be detectable in human plasma and could possibly be a diagnostically sensitive marker of liver pathologies. Indeed, reports of previous studies have described the detection of ALB mRNA in peripheral whole blood and the peripheral mononuclear cell fraction of humans (14–22). These studies have had a mixed...
level of success, however, with detection rates of blood ALB mRNA of <100% from patients with hepatocellular carcinoma (HCC), cirrhosis, or hepatitis and from healthy controls. Yet, Kudo et al. (23) recently reported the presence and correlation of plasma ALB mRNA concentration with hepatic injury in rats.

Blood cells are able to “illegitimately” transcribe genes known to be predominantly expressed by other cell types (24), and we have previously demonstrated that blood cells are the major contributors of nucleic acids in plasma (25). Therefore, we first determined whether ALB mRNA in plasma or whole blood is derived from the liver. After confirming the liver origin of plasma ALB mRNA, we then determined whether quantitative aberrations could be detected in a variety of liver pathologies. To achieve the first aim, we used a previously described RNA–single-nucleotide polymorphism (SNP) strategy (26,27) to genotype ALB mRNA molecules found in the circulation of recipients of liver or bone marrow transplants from donors who were genotypically different for the targeted ALB-coding SNP.

Materials and Methods

Participants
Between June 2006 and April 2008, we recruited participants from the Prince of Wales Hospital, Hong Kong, including (a) patients with a range of liver complications who visited the Departments of Medicine and Therapeutics, and Clinical Oncology, (b) patients who previously had undergone liver transplantation (LT) at the Department of Surgery and the paired living or cadaver donors, (c) patients who had undergone bone marrow transplantation (BMT) at least 6 months earlier at the Department of Pediatrics, and (d) healthy individuals. Ethics approval was obtained from the institutional review board, and informed consent was obtained from all living participants or their responsible guardians.

Ten milliliters of peripheral blood was collected into EDTA-containing tubes. Either buccal cells or hair follicle cells were also collected from the BMT patients. For the participants who had undergone cadaveric LT, archived liver biopsy tissue samples from the deceased donors were retrieved.

Sample Collection and Processing
Immediately after blood collection, samples were kept at 4 °C and processed within 4 h. After gentle mixing of the whole-blood sample, 0.3 mL was mixed with 0.9 mL of TRIzol LS reagent (Invitrogen). Plasma was harvested by a double-centrifugation protocol (28). Details regarding the subsequent processing can be found in the online Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol56/issue1. Buffy coat was isolated after the first centrifugation step and recentrifuged at 230g for 5 min at 4 °C to remove any residual plasma. All samples were stored until nucleic acid extraction, as described in the online Data Supplement.

ALB Genotyping
We targeted a SNP, rs962004, within the ALB coding region. DNA sequencing of 10 unrelated individuals revealed that the SNP has a minor-allele frequency of 0.37. Genotyping was performed by a primer-extension reaction with a homogenous MassEXTEND assay (Sequenom). Extension products for each SNP allele demonstrate distinct masses that can be resolved by MALDI-TOF mass spectrometry analysis (Fig. S1 in the online Data Supplement). Details of the SNP analysis can be found in Table S1 and other information in the online Data Supplement.

Buffy coat DNA was used to determine genotypes of LT recipients and liver donors. For cadaveric LT cases, the ALB genotypes of the deceased donors were determined from the archived liver-biopsy tissue DNA. The original ALB genotypes of BMT recipients were determined from DNA of buccal cells or hair follicles. The ALB genotypes of the bone marrow donors were assessed from theuffy coat DNA of recipients after BMT. All BMT cases were myeloablative, as confirmed by fluorescence in situ hybridization. The same genotyping assay was used with reverse-transcribed ALB mRNA to determine genotypes of ALB mRNA in plasma or whole blood of the transplant recipients.

Quantification of the ALB mRNA Transcript in Plasma
One-step reverse-transcription quantitative real-time PCR (RT-qPCR) was used to measure plasma ALB mRNA concentrations. The intron-spanning assay for ALB mRNA quantification was designed to amplify a 78-bp ALB amplicon across exons 1 and 2 at the 5′ region (GenBank accession no. NM_000477.3); the sequences are summarized in Table S2 in the online Data Supplement. Calibration curves for absolute ALB mRNA quantification were prepared by amplifying serial dilutions of HPLC-purified single-stranded synthetic DNA oligonucleotides (Sigma–Proligo) specific for the targeted ALB amplicon (29) at concentrations...
of 3 copies to $3 \times 10^6$ copies per reaction well. The amplification of ALB mRNA was monitored with an ABI Prism 7900 Sequence Detection System (Applied Biosystems) and Sequence Detection Software version 2.2 (Applied Biosystems). The median PCR efficiency was 88.3% (SD, 6%; range, 81.1%–98.8%), which was calculated from the calibration curves with a median slope of $-3.64$ (SD, 0.18; range, 3.35–3.88), a $y$ intercept at 40.8 (SD, 1.86; range, 38.1–43.5), and a correlation coefficient of 0.9958 (SD, 0.0026; range, 0.9905–0.9986). Absolute concentrations of ALB mRNA in plasma were expressed as the number of copies per milliliter. Details of the RT-qPCR reaction are available in the online Data Supplement.

ASSESSMENT OF LIVER FUNCTION
Plasma analyses for albumin, total bilirubin, alkaline phosphatase, alanine aminotransferase (ALT), and $\alpha$-fetoprotein were performed by the Chemical Pathology laboratory of the Prince of Wales Hospital, Hong Kong, with a DPE Modular Analytics system (Roche Diagnostics). Hepatitis B virus DNA was quantified in the sera of patients with chronic hepatitis B (CHB) infection, as previously described (30, 31). Concentrations $>10,000$ copies/mL were considered evidence for active viral replication (32).

STATISTICAL ANALYSIS
Statistical analyses were performed with SPSS software (version 15.0; SPSS). Plasma ALB mRNA concentrations for the patient and control groups were compared with the Kruskal–Wallis $H$-test, the Mann–Whitney $U$-test, and the Dunn test as appropriate. Correlations between plasma ALB mRNA concentration and other parameters were determined by the Spearman rank correlation. A $P$ value $<0.05$ was considered statistically significant; all probabilities were 2-tailed. An outlier was identified when the plasma ALB mRNA concentration of the sample was $>3$ SDs from the mean of the corresponding group. An ROC curve was constructed to determine the area under the curve. Sensitivity and specificity were calculated at the optimal cutoff plasma concentration of ALB mRNA for distinguishing patients with liver complications from healthy individuals.

Results

ORIGIN OF ALB mRNA IN THE CIRCULATION
To determine if ALB mRNA in plasma and whole blood is liver derived, we developed an RNA-SNP assay to genotype the ALB mRNA molecules found in the circulation of LT and BMT recipients. We focused our analysis on informative donor–recipient pairs, i.e., pairs in which the donor bore a genotype for the interrogated ALB SNP different from that of the corresponding recipient. After LT, the genotype corresponding to that of the donor should be observed for the ALB mRNA molecules found in the circulation of the recipient if the ALB mRNA is genuinely liver derived. Alternatively, if other tissue sources contributed to the pool of circulating ALB mRNA, the ALB genotype of the recipient should be detectable. To demonstrate that hematopoietic cells could be a contaminating source of circulating ALB mRNA, we performed a similar RNA-SNP analysis for BMT recipients. Similarly, the circulating ALB mRNA molecules would exhibit the genotype of the bone marrow donor if hematopoietic cells contributed ALB mRNA to the circulation.

We studied 29 LT cases. Nine of the recipients obtained their livers from living relatives; the rest received their livers from cadavers. Fifteen of these donor–recipient pairs were deemed informative in that the donor and recipient showed distinctive ALB genotypes that allowed subsequent genotype comparison. Table 1 summarizes the genotyping data for the informative donors and recipients. Among the informative cases, the ALB mRNA genotypes detected in the plasma of recipients after transplantation were different from their original genotypes and corresponded to those of the liver donors.

Five of the 20 BMT cases recruited were informative; the genotyping data are summarized in Table 1. There was no change in the recipients’ ALB mRNA genotypes in plasma before and after BMT. Thus, the donor’s bone marrow was not a major contributor of ALB mRNA in the recipient’s plasma.

Besides plasma, RNA-SNP analysis was also performed on ALB mRNA from whole blood collected from the informative LT and BMT recipients after transplantation. The patients in 12 informative LT cases and 4 informative BMT cases consented to this analysis; the genotyping data are shown in Table 1. Unlike the plasma data, contributions from the bone marrow donors (cases B8 and B10) and the LT recipients (cases L8, L18, L23, L24, and L25) were observed in the ALB mRNA from whole blood. In summary, plasma ALB mRNA was liver derived, whereas ALB mRNA in whole blood was not liver specific.

QUANTITATIVE ANALYSIS OF PLASMA ALB mRNA

LT RECIPIENTS AND HEALTHY CONTROLS
After confirming that plasma ALB mRNA was liver specific among the transplant recipients, we assessed its concentration in plasma samples from the LT recipients and 207 healthy controls. Of the 29 LT recipients who underwent LT in the Prince of Wales Hospital, Hong Kong, 24 continued to make follow-up visits in the same hospital. Fifteen of these 24 recipients remained well, with typical profiles in liver-function
tests. We compared the plasma \textit{ALB} mRNA concentrations in these 15 recipients and the controls. The data are summarized in Fig. S2 in the online Data Supplement. The 2 groups were not significantly different ($P = 0.686$, Mann–Whitney $U$-test).

**PATIENTS WITH LIVER DISEASES AND HEALTHY CONTROLS**

We then assessed \textit{ALB} mRNA concentrations in plasma samples obtained from 107 patients with a spectrum of liver complications and compared the results with those for the same 207 healthy individuals described above. Among the patients, 35 were confirmed to have HCC, 25 had biopsy-proven liver cirrhosis, 24 had CHB with serum concentrations of hepatitis B virus DNA $>10,000$ copies/mL (i.e., active viral replication), and 23 had CHB with serum concentrations of hepatitis B virus DNA $<10,000$ copies/mL (i.e., inactive viral replication). All healthy individuals tested negative for hepatitis B surface antigen and had values in liver-function tests that were within the reference intervals for plasma.

Information about demographics, biochemical testing, virologic investigations, and the participants’ median plasma \textit{ALB} mRNA concentrations are summarized in Table 2. Plasma \textit{ALB} mRNA was detected in 308 of the 314 participants (98.1%), with negative readings for 1 patient and 5 controls. Fig. 1 shows the plasma \textit{ALB} mRNA concentrations for all the groups. The participant groups had significantly different \textit{ALB} mRNA concentrations ($P = 0.0001$, Kruskal–Wallis $H$-test). A subgroup analysis showed that patients with HCC ($P = 0.0001$), liver cirrhosis ($P = 0.0068$), or active CHB ($P = 0.0001$) had significantly increased plasma \textit{ALB} mRNA concentrations compared with controls. There was no statistically significant difference in plasma mRNA concentration between the controls and patients with inactive CHB ($P = 0.4239$, Dunn test).

ROC curve analysis was performed to assess whether the plasma \textit{ALB} mRNA concentration is an effective indicator of liver pathologies (Fig. 2). The area under the ROC curve suggested that the diagnostic efficacy of plasma \textit{ALB} mRNA as an indicator of liver pathologies was 92.9%. Use of a plasma \textit{ALB} mRNA cutoff of 835 copies/mL yielded a diagnostic sensitivity of 85.5% and a diagnostic specificity of 92.8% for detecting any one of the assessed liver pathologies.

### Table 1. \textit{ALB} genotypes of the informative transplant recipients and donors.

<table>
<thead>
<tr>
<th>Transplant type</th>
<th>Case no.</th>
<th>Original genotype of recipient</th>
<th>Genotype of donor</th>
<th>Posttransplantation genotype in plasma of recipient</th>
<th>Posttransplantation genotype in whole blood of recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT L1</td>
<td>A</td>
<td>AG</td>
<td>AG</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>LT L4</td>
<td>A</td>
<td>AG</td>
<td>AG</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>LT L6</td>
<td>G</td>
<td>AG</td>
<td>AG</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>LT L8</td>
<td>AG</td>
<td>A</td>
<td>A</td>
<td>AG</td>
<td></td>
</tr>
<tr>
<td>LT L9</td>
<td>AG</td>
<td>A</td>
<td>A</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>LT L11</td>
<td>A</td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
<td></td>
</tr>
<tr>
<td>LT L12</td>
<td>A</td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
<td></td>
</tr>
<tr>
<td>LT L18</td>
<td>AG</td>
<td>A</td>
<td>A</td>
<td>AG</td>
<td></td>
</tr>
<tr>
<td>LT L20</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>LT L22</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>LT L23</td>
<td>AG</td>
<td>A</td>
<td>A</td>
<td>AG</td>
<td></td>
</tr>
<tr>
<td>LT L24</td>
<td>AG</td>
<td>A</td>
<td>A</td>
<td>AG</td>
<td></td>
</tr>
<tr>
<td>LT L25</td>
<td>AG</td>
<td>—</td>
<td>G</td>
<td>AG</td>
<td></td>
</tr>
<tr>
<td>LT L27</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>AG</td>
<td></td>
</tr>
<tr>
<td>LT L29</td>
<td>G</td>
<td>AG</td>
<td>AG</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>BMT B1</td>
<td>AG</td>
<td>A</td>
<td>AG</td>
<td>AG</td>
<td></td>
</tr>
<tr>
<td>BMT B8</td>
<td>A</td>
<td>AG</td>
<td>A</td>
<td>AG</td>
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<td>BMT B9</td>
<td>AG</td>
<td>A</td>
<td>AG</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>BMT B10</td>
<td>AG</td>
<td>G</td>
<td>AG</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>BMT B13</td>
<td>AG</td>
<td>G</td>
<td>AG</td>
<td>AG</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ A, homozygous A/A genotype; G, homozygous G/G genotype; AG, heterozygous A/G genotype; —, data not available.

$^b$ The donor genotype for case L25 was not known because the patient underwent liver transplantation in mainland China.
RELATIONSHIP BETWEEN PLASMA ALB mRNA CONCENTRATION AND CONVENTIONAL LIVER-FUNCTION TEST PARAMETERS

Considering the data from all of the study participants (patients and controls) showed that plasma ALB mRNA concentration weakly correlated with plasma total bilirubin ($r = 0.133; P = 0.018$, Spearman correlation), alkaline phosphatase ($r = 0.126; P = 0.0255$), and ALT ($r = 0.207; P = 0.0002$) (Fig. 3).

Of 107 patients, only 23 patients (21.5%) had increased ALT activities (58 U/L), whereas 62 patients (73.8%) had plasma ALB mRNA concentrations >835 copies/mL. For the 35 patients with HCC confirmed by liver biopsy, only 17 patients (48.6%) had increased $\alpha$-fetoprotein concentrations (>20 $\mu$g/L) (33); however, 32 (91.4%) of these patients had increased plasma ALB mRNA concentrations (Fig. 4).

**Discussion**

There is much excitement about the possibility of developing blood-based tools for disease diagnosis and management through the analysis of circulating nucleic acids (2–6). The detection of circulating RNA offers certain advantages over the detection of circulating DNA (24). Because the expression profiles of cell types and diseases are different, tissue- or disease-specific transcripts could be exploited as markers for disease assessment. If an RNA transcript unique to a particular organ is selected, the RNA approach may be more generally applicable to diseases of that organ and not be limited to the fraction of patients harboring specific DNA signatures. Furthermore, if both plasma RNA and DNA were derived from the same cell population, the released RNA would likely be quantitatively more abundant than DNA. This is because multiple copies of an RNA transcript may be present in each cell, depending on the gene’s expression, whereas each cell contains only a single diploid genome equivalent of DNA. Indeed, some cancer researchers reported that a greater proportion of cancer cases were positive for the investigated plasma RNA markers than DNA markers (3).

An increasing amount of evidence suggests that liberation of cell-free nucleic acids into plasma from organs or compartments is likely to be due to cell death (13, 34, 35). The liver being one of the largest organs of the body, we suspect that RNA expressed by genes in the liver, such as ALB, should be detectable in the peripheral circulation because of cell death associated with typical cell turnover and/or with pathologic damage. Indeed, studies have reported the presence of circulating ALB mRNA, but with varying degrees of success (14, 15, 17, 18, 21, 36). Some researchers have suggested that ALB mRNA in blood originates from malignant or nonmalignant hepatocytes that have entered the peripheral circulation (14, 15, 18, 20); however, Muller et al. (16) reported that peripheral mononuclear cells can be induced to produce ALB mRNA. Indeed, previous reports have indicated that certain

### Table 2. Profile of the study participants.

<table>
<thead>
<tr>
<th>Group</th>
<th>HCC</th>
<th>Cirrhosis</th>
<th>Active CHB (HBV&lt;sub&gt;x&lt;/sub&gt; DNA ≥10 000 copies/mL)</th>
<th>Inactive CHB (HBV&lt;sub&gt;x&lt;/sub&gt; DNA &lt;10 000 copies/mL)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals, n</td>
<td>35</td>
<td>25</td>
<td>24</td>
<td>23</td>
<td>207</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n</td>
<td>33 (94%)</td>
<td>19 (76%)</td>
<td>20 (83%)</td>
<td>17 (74%)</td>
<td>141 (68%)</td>
</tr>
<tr>
<td>Female, n</td>
<td>2 (6%)</td>
<td>6 (24%)</td>
<td>4 (17%)</td>
<td>6 (26%)</td>
<td>66 (32%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>55 (10)</td>
<td>61 (9)</td>
<td>43 (12)</td>
<td>47 (11)</td>
<td>45 (10)</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>42 (23–48)</td>
<td>39 (26–47)</td>
<td>44 (39–50)</td>
<td>48 (42–49)</td>
<td>46 (40–52)</td>
</tr>
<tr>
<td>Bilirubin, µmol/L</td>
<td>12 (6–50)</td>
<td>24 (3–188)</td>
<td>12 (4–34)</td>
<td>13 (5–25)</td>
<td>13 (2–29)</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>86 (43–147)</td>
<td>83 (47–210)</td>
<td>70 (37–111)</td>
<td>68 (40–101)</td>
<td>65 (32–114)</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>41 (21–317)</td>
<td>40 (14–197)</td>
<td>37 (12–73)</td>
<td>25 (10–64)</td>
<td>21 (10–58)</td>
</tr>
<tr>
<td>Hepatitis B surface antigen, n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>29</td>
<td>18</td>
<td>24</td>
<td>23</td>
<td>0</td>
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<tr>
<td>Negative</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>207</td>
</tr>
<tr>
<td>Not known</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plasma ALB mRNA, copies/mL</td>
<td>3500 (100–57 000)</td>
<td>2500 (0–43 000)</td>
<td>3600 (160–1 500 000)</td>
<td>1600 (100–8 000)</td>
<td>200 (0–2000)</td>
</tr>
</tbody>
</table>

* Age data are presented as the mean (SD). Data for albumin, bilirubin, alkaline phosphatase (ALP), ALT, and plasma ALB mRNA concentration are presented as the median (range). HBV, hepatitis B virus.
supposedly organ-specific transcripts detectable in the circulation may in fact be derived from other cell populations, such as hematopoietic cells (27, 37), owing to illegitimate gene transcription (24, 38).

Thus, our first aim in this study was to confirm whether ALB mRNA detectable in human plasma and whole blood is derived from and specific to the liver. We studied LT and BMT donor–recipient pairs of individuals who were genotypically different with respect to an ALB-coding SNP and determined the RNA-SNP genotypes in plasma and whole blood. Our data have demonstrated that the ALB mRNA detected in plasma is liver specific, but not that in whole blood. The data also indicated that ALB mRNA produced by hematopoietic cells could contribute to the pool of ALB mRNA detected in whole blood. These findings call for caution in the interpretation of the previously reported data on ALB mRNA detection in whole blood (14, 15, 17, 18).
Plasma is to be preferred over whole blood for future studies of ALB mRNA as a biomarker for liver diseases. To minimize the chance of residual blood cells contaminating the ALB mRNA molecules in plasma, we recommend that plasma be prepared by 2 centrifugation steps, as previously reported (28).

We then developed a 1-step RT-qPCR assay for plasma ALB mRNA quantification. The overall detection rate for plasma ALB mRNA in our study was 97.6%. A recent study used 2-step RT-qPCR to investigate the role of plasma ALB mRNA detection for predicting HCC recurrence in LT recipients and reported a detection rate of 82% (36). The authors of the report believed that the absence of detectable ALB mRNA in the plasma of the study participants indicated a lack of HCC cells in the circulation. We have shown, however, that plasma ALB mRNA is detectable both in LT recipients despite the lack of development of HCC and in almost all other persons, including healthy controls. These data indicate that circulating malignant cells from the liver are not the sole source of ALB mRNA in the plasma. Our improvement in detection rate may be related to the analytical sensitivity of our 1-step RT-qPCR protocol and our targeting more toward the 5’ end of the ALB gene. We have previously reported that circulating mRNA in plasma may not be intact full-length transcripts but instead may be predominantly 5’ fragments (29).

We found that the plasma ALB mRNA concentrations of healthy control individuals and the LT recipients who remained well were not significantly different. These data suggest that our findings of the liver origin of plasma ALB mRNA proved through the study of LT recipients should be applicable to non-LT individuals. Patients with HCC, cirrhosis, and active CHB (but not those with inactive CHB) had significantly higher plasma ALB mRNA concentrations than controls. These data suggest that ALB mRNA may be released into the plasma upon cell death, and therefore the concentration may correlate with the degree of cell death. Thus, future studies should assess whether the plasma concentration of ALB mRNA is prognostic for liver diseases.

Our ROC curve analysis demonstrated plasma ALB mRNA measurement to be an attractive means for detecting the presence of liver pathologies (91.9% of patients). In particular, for the HCC group, α-fetoprotein was increased in only 48.6% of the cases, whereas the majority (91.4%) of these patients showed increased ALB mRNA concentrations.

Plasma ALB mRNA concentration showed some correlation with serum ALT activity. Plasma ALB mRNA was also increased in patients who had liver disease but nonpathologic ALT values (Fig. 3). These observations suggest that plasma ALB mRNA may be a diagnostically sensitive marker for detecting early-stage chronic liver disease, when the ALT activity or concentration is often within the reference interval. Further investigation is warranted.

In summary, our data have revealed that ALB mRNA in plasma, but not in whole blood, is derived from and specific to the liver. Plasma ALB mRNA concentrations were increased for a range of liver pathologies; further studies are required, however, to investigate the clinical utility of this marker for assessing or managing such diseases.

References
Liver Pathologies and Plasma ALB mRNA

122–30.