Table 1: Evaluation of serum alpha fetoprotein as a marker of circulation tumor cell in different hepatocellular carcinoma studies. nRT-PCR, nested RT-PCR; qRT-PCR, quantitative RT-PCR.

Author	PCR	Sensitivity	Cases	Samples	Positivity	Predictability of recurrence
[55]	qRT-PCR	1 CHC/10^7	37	Blood	18%	No
			136	BM	28%	Yes
[64]	qRT-PCR	1 CHC/10^6	38	Blood	10%	Yes
			25	BM	48%	No
[56]	nRT-PCR	5 cells/1 mL	24	Blood	29%	Suspect
				BM	43%	
[54]	Competitive RT	10 cells/9 mL	22	Blood	26%	NA
			11	BM	45%	
[90]	RT-PCR	NA	18	BM	93%	No
[61, 62]	nRT-PCR		33	Blood	52%	Extrahepatic metastases
[57]	nRT-PCR	15 cells/mL	64	Blood	36%	Extrahepatic metastases
[58]	nRT-PCR	1CHC/10^5 mono	20	Blood	25%	No
[91]	nRT-PCR	10^{-6} μg/μL of RNA	33	Blood	54%	Yes
[92]	nRT-PCR	1CHC/10^5 mono	87	Blood	36%	Yes
[93]	nRT-PCR	1 CHC/10^7 mono	85	Blood	26–45%	No
[88]	RT-PCR	1 CHC/10^6	52	Blood	25%	No

take advantage of the potential for stem cell support of the BM microenvironment. The amplification of AFP mRNA by means of reverse transcription (RT) and a nested polymerase chain reaction (PCR) is the highly sensitive method for the detection of residual HCC cells in peripheral blood. The qualitative (positive versus negative) detection of HCC circulating tumor cells in blood samples from individual patients is of limited value in predicting the risk of disease progression. Because the level of AFP mRNA is increased in HCC tissue compared with in normal hepatocytes, the quantification of AFP transcripts seems to be a more reliable indicator of disease progression. A more highly sensitive assay based on TaqMan technology to quantify AFP mRNA in "real time" should be preferred [59, 87–89]. Even using this methodology, reported results are not homogeneous and contradictory [72]. The main studies which have evaluated AFP mRNA are summarized in Table 1. The false-positive results can be obtained using AFP mRNA.

2.3. Alpha Albumin (ALF). For more than a decade, we know that mRNAs of hepatocytes-specific albumin genes are detected in peripheral blood by RT-PCR. It was shown that there is evidence that detection of albumin mRNA associated with the detection of AFP mRNA is strongly associated with the presence of metastases [57, 61, 94, 95]. Wong et al., showed that circulating hepatocellular carcinoma cells can be detected and be semiquantified by albumin RT-PCR [96]. On the other hand, Resto et al., showed that downregulation of alpha-albumin (ALF) specifically in HCC-circulating cells can be used as a specific marker to discriminate the normal hepatocellular circulating cells that express abundantly ALF. RT-PCR ALF in association with RT-PCR AFP have been proposed to distinguish normal or malignant hepatocytes in peripheral blood, but the interpretation of the results is still debated [97].

2.4. Transforming Growth Factor Beta-1 (TGF-β1). The levels of circulating TGF-β1 and TGF-β1 mRNA were significantly higher in the HCC patients than any other group of patients. The sensitivity and specificity of circulating TGF-β1 level (>1.2 μg/L) were 90% and 94% for HCC diagnosis, but no significant correlation was found between TGF-β1 expression an AFP levels or tumor size. The combined detection of TGF-β1 and serum AFP could raise the detection rate of HCC up to 97%. Both of circulating TGF-β1 and TGF-β1 mRNA could be used as sensitive biomarkers for diagnosis and prognosis of HBV-induced HCC [98, 99]. Unfortunately, TGF-β1 mRNA was poorly studied and further investigations have to be done to use circulating TGF-β1 mRNA as a marker of circulating tumor cells in HCC.

2.5. Insulin-Like Growth Factor (IGF)-II. Studies using amplified fragments of IGF-II mRNA by RT-PCR showed that the lowest sensitivity with 2 ng/L of total RNA. Dong et al., showed that the positive frequencies of IGF-II mRNA were 100% in HCC, around 50% in paracancerous and 0% in noncancerous tissues respectively. But, the positive frequency of circulating IGF-II mRNA was 34% in HCC, and no amplification was found in other liver diseases, extrahepatic tumors, and normal control, meaning that IGF-II is specific of the HCC but not really sensitive. Associated to other circulating markers IGF-II can be helpful to detect CTC. The circulating IGF-II mRNA was correlated with the stage of HCC (incidence = 100%) with extrahepatic metastasis and 35% with AFP-negative. No difference was found between tumor size and circulating IGF-II mRNA [99, 100] but these results are controversial [101].

2.6. Prostate-Specific Antigen (PSA). The Prostate-Specific Antigen (PSA) had shown to be well-established reliability marker and remained a valid prostate marker in patients