Further evidence on the underestimation of the prevalence of TT viral DNA in blood donors

We reassessed the prevalence of TT viral DNA in Italian blood donors by using primers derived from a highly conserved non-coding region of the viral genome. Our previous underestimation of the prevalence of TTV is proved by the new figure obtained: 85% vs 5%. Again, we observed no difference with respect to a group of potential donors with elevated alanine aminotransferase levels.

Sir,

We recently reported a 5% prevalence of TTV DNA in Italian blood donors. This prevalence was not significantly different with respect to that in a group of potential donors with elevated serum alanine aminotransferase (ALT) levels. At the time we started our investigation, data reported from all over the world were showing not only TTV infection to be widespread in the normal population but also to have a geographical distribution. In fact, by using primers derived from the N22 clone, a wide range of TTV prevalence had been observed, as shown in Table 1. This region of the viral genome was being widely employed since it allowed phylogenetic analysis to be carried out. Such analysis had revealed a marked genome heterogeneity of the virus and the existence of several TTV types and subtypes. In our study we used two sets of semi-nested primers recognizing an internal region of the N22 clone and observed, by sequencing the amplified products, a comparable degree of sequence variability. On this basis, we felt that the portion of the viral genome corresponding to clone N22 was not suitable for detecting all variants of the virus and that therefore factors other than geographical differences were contributing to the wide range of TTV prevalence worldwide, especially to the discrepancies in TTV frequency observed within the same country, as in the case of Thailand and Italy. With respect to Japanese blood donors, Takahashi et al. had already pointed out the importance of using an appropriate set of conserved primers. In fact, when they selected a pair of primers (T801/T935) that specifically amplifies a portion of a non-coding region of the viral genome spanning nucleotides 6-204 of the prototype isolate TA278, they detected TTV DNA in 92% of healthy adults as opposed to 12% determined earlier for blood donors in Japan. More recently, Leary et al. confirmed, by using three novel sets of nested primers amplifying highly conserved non-coding regions, that TTV infection in the human population has so far been underestimated. These findings prompted us to re-evaluate the prevalence of TTV DNA in the same 500 Italian blood donors by using the set of nested primers proved by Leary et al. to be the most efficient one. The region amplified is comprised between nt 3087 and nt 3392 of the TA278 sequence. The overall prevalence resulted to be 85±3% (425/500) with all the previously positive samples confirmed as positive. No statistically significant difference was observed with respect to the prevalence in the 95 potential donors with elevated ALT levels (79±8%). The specificity of the amplification was confirmed by sequencing the polymerase chain reaction (PCR) products and by comparing the sequences with those stored in databanks. Although the role of TTV in human liver disease is still unclear, our results once again point to the dubiousness of TTV having a role in unexplained hepatitis. The dramatic reversal of the previously estimated TTV prevalence in blood donors as shown here and by other groups confirms that caution should be used whenever employing PCR to study the epidemiology of viral infections, the choice of primers, in terms of specificity and sensitivity, being of crucial importance.

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Key words
TTV DNA; blood donors; ALT.

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References

Karyotype refinement in five patients with acute myeloid leukemia using spectral karyotyping

Five patients with acute myeloid leukemia showing several complex rearrangements were examined by spectral karyotype (SKY) analysis. Twenty rearrangements, misclassified or undetected by standard cytogenetic techniques, were identified. The present study supports the ability of SKY to detect the organization of chromosome rearrangements and to identify new prognostic markers.