References

1. Guidelines for thromboplastins and plasmas used to control anticoagulant therapy. World Health Organization Committee on Biological Standardization. WHO Technical Report Series 1999; 889:64-93.
2. van den Besselaar AM, Breddin K, Lutze G, Parker-Williams J, Taborski U, Vogel G, et al. Multicenter evaluation of a new capillary blood prothrombin time monitoring system. Blood Coagul Fibrinolysis 1995; 6:726-32.
3. Taborski U, Müller-Berghaus G. State-of-the-art patient self-management for control of oral anticoagulation. Semin Thromb Hemost 1999; 25:43-7.
4. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1:307-10.
5. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. J Clin Chem Clin Biochem 1983; 21:709-20.
6. Cosmi B, Palareti G, Carpanedo M, Pengo V, Biasiolo A, Rampazzo P, et al. Assessment of patient capability to self-adjust oral anticoagulant dose: a multicenter study on home use of portable prothrombin time monitor (COAGUCHECK). Haematologica 2000; 85:826-31.

Reliability of measurements of serum alanine transaminase activity and the impact on the cut-off value for the selection of blood donors

The alanine-transaminase (ALT) threshold for screening blood units is not homogeneous in italian blood centers and this phenomenon produce a great variability in the donor-acceptance rate. The standardization of ALT cut-off level, unifying the statistical methods to calculate the threshold of acceptance, would decrease the variability between centers.

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Before the discovery of hepatitis C virus (HCV) and development of the HCV assay, serum alanine transminase (ALT) levels were used to identify donors potentially infected with non-A non-B hepatitis.[1] In some countries, transfusion centers (TC) continue to use ALT testing in screening donors, despite this practice being controversial[1,2] in that although it reduces the residual risk of post-transfusional hepatitis,[1,3-4] it decreases the donation acceptance-rate. In Italy, although ALT levels continue to be used in blood screening, the regulations governing their use are insufficient,[5,6] and the existing guidelines[7] for determining ALT cut-off levels are not mandatory. Consequently, blood donated by persons with the same ALT level can be accepted by one TC yet rejected by another.

We conducted a study to describe the variability among TCs with respect to the methods used for measuring serum ALT levels and for calculating the cut-off levels for accepting blood donations, in order to evaluate the impact of these factors on the donation-acceptance rate.

Nine TCs in Italy participated in the study, providing information on the assay used to measure ALT levels, the cut-off ALT level adopted, and the method used to calculate this level. The TCs analyzed, in duplicate, serum samples taken from the same 20 blood donors (M/F: 14/6; mean age: 42 years; range: 18-50 years) with ALT levels slightly higher than the normal level [1.1-

Table 1. Relationship between the ALT measurement of each transfusion center (TC) and the reference center (TC no. 2).

TC	*Regression model* $y_i^* = a + b \times_2 °$	*Correlation coefficient*	*Standardized ALT cut-off level#*
1	$y_1 = 13.19 + 1.11 \times_2$	0.98	73.47
2	$y_2 = x_2$	–	54.31
3	$y_3 = - 2.79 + 1.05 \times_2$	0.99	54.23
4	$y_4 = - 2.44 + 1.17 \times_2$	0.99	61.10
5	$y_5 = - 1.85 + 0.95 \times_2$	0.99	49.74
6	$y_6 = 0.49 + 0.93 \times_2$	0.99	51.00
8	$y_8 = 0.11 + 1.21 \times_2$	0.99	65.82
9	$y_9 = -3.41 + 1.08 \times_2$	0.99	55.24

**y_i=TC$_i$ ALT value; °$\times_2$ = TC$_2$ ALT value; #by regression model.*

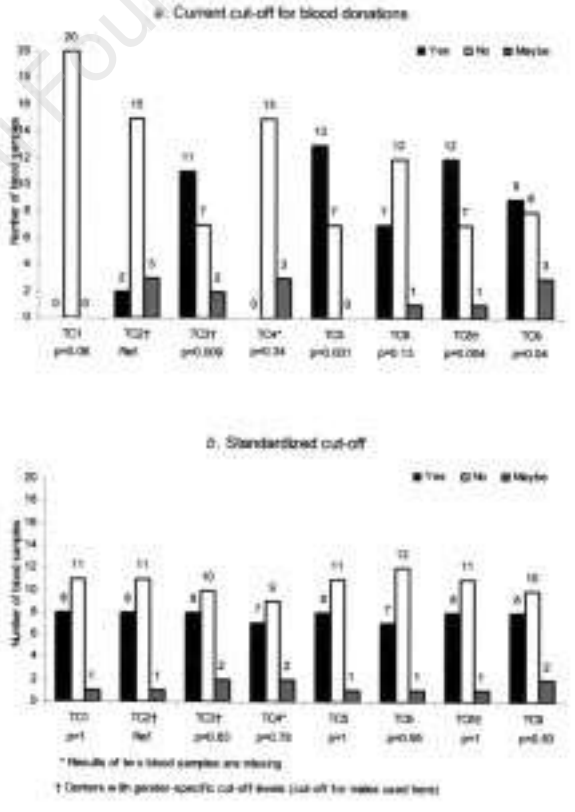

Figure 1. Classification of the suitability of blood samples (n=20) for donation by transfusion center (TC) and the statistical significance of the difference between each TC and the reference center (TC no. 2). The classification of the suitability of blood samples for donation was based on two repeated ALT measurements. The classification categories were: *yes*, if both results were lower or equal to the cut-off level; *no*, if both were higher, and *maybe* if discordant.

2.3 times the maximum normal value of TC no. 2, which was selected as the reference center]. These levels were chosen because, in practice, they constitute the most problematic range. Based on their routine procedures, the TCs decided whether or not to accept the corresponding blood donation.

The results of the study revealed that the nine TCs use seven different commercial assays to measure ALT levels, although these assays are based on 2 biochemical methods. The TCs use 5 different statistical methods to calculate the ALT cut-off level, which varies considerably among TCs, ranging from 45.0 to 77.7 U/L for males and from 40.0 to 60.0 U/L for females. As shown in Figure 1a, the donor-acceptance rate also varies greatly among TCs when using the current ALT cut-off levels, with the greatest difference observed between TC #1, which accepted none of the 20 samples, and TC #5, which accepted 13 of the samples ($p < 0.00001$). These differences were also observed when considering those TCs with different cut-off levels for males and females.

The high intra-laboratory reliability (intra-class correlation coefficient ≥ 0.95 of 20 matched sample pairs) and the high inter-laboratory correspondence (linear correlation coefficient = 0.98-0.99; Table 1) suggest that biochemistry was not a relevant source of variability.

The variability among the TCs greatly decreased (Figure 1b) when linear regression models were used to define cut-off levels standardized for biochemical methods using as reference an intermediate value of 51.0 U/L, which corresponds to the cut-off value for TC #6 (Table 1). Specifically, all TCs would accept 8 of the 20 samples, and the number of samples that would be rejected ranged from 10 to 12. Moreover, the percent agreement between TCs in classifying samples increased (*data not shown*).

In conclusion, the use of different ALT cut-off levels in screening blood units can produce great variability in the donor-acceptance rate. However, in interpreting these data, it should be taken into consideration that all of the samples had ALT levels slightly higher than the normal level, when the greatest differences in the acceptance rate are expected: in actual practice, the variability would probably be lower. Furthermore, to mimic the effect on the donor-acceptance rate of a single statistical method to define the cut-off level, we used an intermediate reference value of 51 U/L, although we do not suggest adopting just one cut-off value standardized for biochemistry because we do not have data to verify that differences in the distribution of ALT levels among populations are negligible.

Unifying the statistical methods to calculate ALT cut-off levels would be a first step in decreasing the variability in donor-acceptance rates, while taking into account the epidemiological characteristics of individual reference populations. In fact, the absence of standardized methods for calculating these levels reduces the potential benefits of ALT testing. This is particularly important in the light of the finding that approximately 20% of blood donors who are negative for HBsAg and HCV antibodies have ALT levels that exceed 40 U/L.[8] Although the forthcoming introduction of nucleic acid testing technology for blood screening will be useful in identifying occult infections, and consequently will increase the safety of the blood supply, it will still be necessary to determine whether non-transmissible hepatic disorders (e.g., celiac disease, non-alcoholic fatty liver disease) can explain all residual cases of increased ALT levels, in order to evaluate the cost-effectiveness of ALT screening more accurately.

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References

1. Infectious Disease Testing for Blood Transfusion. NIH Consensus Conference. JAMA 1995; 274:1374-9.
2. Blajchman MA, Bull SB, Feinman SV. Post-transfusion hepatitis: impact of non-A, non-B hepatitis surrogate tests. Lancet 1995; 345:21-5.
3. Schreiber GB, Busch MP, Kleinman S. Transfusion-transmitted viral infection. N Engl J Med 1996; 26:1685-90.
4. Mele A, Stroffolini T, Catapano R, Palumbo F, Moiraghi A, Novaco F. Incidence of transfusion associated B and non-A, non-B hepatitis in Italy. BMJ 1995; 311:846-7.
5. Decreto del Ministero della Sanità 21.07.1990. Published in "Gazzetta Ufficiale della Repubblica Italiana" n. 195 of the 22.08.1990.
6. Decreto del Ministero della Sanità 15.1.1991. Published in "Gazzetta Ufficiale della Repubblica Italiana" n. 20 of the 24.01.1991
7. de Stasio G, Mannella E, Lanteri M e Gruppo Collaborativo Malattie Trasmissibili con la trasfusione della SIMTI. Linee Guida per la prevenzione delle malattie infettive trasmissibili con la trasfusione. Il Servizio Trasfusionale n.3 (maggio-giugno) 1995.
8. Morisco F, Di Lonardo A, Stroffolini T, Leone D, Caporaso N. High prevalence of non-virus/ non-alcohol-related alanine-aminotransferase increase in blood donors. Haematologica 2001; 86:1116.