CYTOMEGALOVIRUS SEROLOGICAL SCREENING IN BLOOD DONORS

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INTRODUCTION

Like other members of the Herpesviridae family, **Human Cytomegalovirus** (HCMV) can remain latent in some blood cells (B and T lymphocytes, polymorphonuclear leucocytes). CMV infection is endemic and ubiquitous, affecting 40-80% of the population in industrialized countries and almost all the population in developing countries.

In some categories of patients (immunocompromized patients, organ and bone marrow recipients, cancer (oncologic) patients, patients with AIDS) CMV acts as an opportunistic pathogen, causing multi-organ involvement (lungs, liver, intestine, central nervous system, etc.) with high mortality rates (up to 60-80% without antiviral treatment). Serologically negative organ recipients can contract the infection from an organ from a CMV-positive donor.

As regards blood transfusions, theoretically blood from all seropositive donors can transmit the infection, as it contains cells carrying the latent infection: the risk of infection is correlated to the volume of transfused blood and the number of donors. In transfused seronegative children, the probability of contracting the infection is 50% if the donor is CMV-seropositive.

The use of seronegative blood for transfusions to seronegative patients is used as a means of prevention; however, the limited availability of blood from seronegative donors for use with high-risk patients has led to an increasing interest in the development of methods to eliminate the infectivity of blood from seropositive donors.

The effectiveness of leucodepletion (bedside, laboratory and pre-storage filtration) is now widely documented, and reduces the number of white blood cells present in the blood components from 3 to 5 log10 (99.9%).

In clinical terms, leucodepletion not only prevents and reduces the risk of viral reactivation/transmission in the recipient but is also effective in preventing febrile non-hemolytic transfusion reaction, HLA alloimmunization and consequent platelet refractoriness and acute graft-versus-host rejection (GvHD), above all in immunosuppressed patients.

As regards CMV, the available literature describes several studies which state that leucodepletion is as effective as serological screening in reducing the risk of infection transmission.

MATERIALS AND METHODS

Since January 1996 at the Central Regional Virology Laboratory and Molecular Biology of Ancona Hospital (Regional Department of Transfusion Medicine), serological screening has been in place to research specific anti-CMV IgM and IgG antibodies in the donated blood units.

The serological screening protocol includes:

- screening for anti-CMV IgM and IgG in candidate donors attending one of our donor centers for the first time, for serological screening for the virus
- screening for anti-CMV IgM and IgG in periodic CMV-negative donors, on each donation, to highlight any seroconversion, leading to the elimination of the blood unit and the temporary suspension of the donor;
- screening only for anti-CMV IgM in periodic donors already testing positive for IgG, on each donation, to highlight any recurrent or reactivated infection.

In the period of study from 1st January 2013 to 30th April 2014 we analyzed:

- A total of 167,441 samples (136,770 donations of blood and blood components, 20,065 annual controls of periodic donors and 10,606 controls of willing donors) to screen for specific class anti-CMV IgM antibodies using CMIA and ECLIA immunometric assay methods.
- A total of 53,807 samples (donations of blood and blood components and annual controls of seronegative donors) to screen for specific class anti-CMV IgG antibodies using the CMIA immunometric assay method.
- Doubt or positive samples were re-tested with the LIAISON® XL system (DiaSorin Saluggia Italy): 6% of the samples were tested with the LIAISON® XL CMV II IgM test and 10% of the samples with LIAISON® XL CMV II IgG (DiaSorin Italy).

RESULTS

CMV-IgM Pos	2013	Jan-Apr 2014	TOTAL	%
Candidate donors	75	23	98	0.92
Annual control of periodic donors	143	58	201	1.0
Donations	134	31	165	0.12
TOTAL	352	112	464	0.28

Tab 1: Samples confirmed CMV-IgM positive during the study

CMV IgM Pos donations	2013	Jan-Apr 2014	Total
N° Donors with primary CMV infection (previous IgG neg)	97	21	118
N° Donors with CMV reinfection/ reactivation (previous IgG pos)	37	10	47
Total	134	31	165

Tab 2: Donations of CMV-IgM positive blood components and blood

None of the acceptance and donation consent forms (required under Ministerial Decree - D.M. of 3rd March 2005) completed by the periodic donors who were CMV-IgM positive reported any symptoms.

The 134 confirmed CMV-IgM positive blood and blood component donations corresponded to:

- 118 units of blood from seronegative periodic donors (with previous IgG-CMV control negative) and therefore with a primary infection in progress (Tab 1);
- 47 units of blood from periodic donors with previous IgG-CMV control positive and therefore with viral reactivation or reinfection (Tab 2).

IgG CMV Negative	N° IgG CMV negative 2013	% IgG CMV negative	N° IgG CMV Negative Jan-Apr 2014	% IgG CMV negative
Periodical donors 43763 in 2013 28480 in 2014	12870	29.4	8407	29.5
Candidate donors 3696 in 2013 1798 in 2014	1224	33.1	619	34.4

Tab 3. Prevalence of IgG-CMV negative donors

These percentages match the estimates described in literature for the Italian population, in which approximately 30% of healthy people in the same age group (18-60) results CMV seronegative.

CONCLUSIONS

Two approaches have been found to be particularly effective in preventing the transmission of the CMV infection through transfusion: the first is based on the administration of units of blood from seronegative donors to seronegative recipients; the second is based on the administration of units of leucocyte-depleted blood and blood components using leucodepletion filters, which are of proven efficacy but rather costly. The approach we chose involves the selection of donors by serological screening to test for anti-CMV antibodies, in order to prevent transmission of the infection to groups of recipients at risk (immunocompromized patients, cancer patients, organ recipients, children, newborns, CMV-negative pregnant women) who suffer more frequently and more seriously from complications caused by the CMV infection than immunocompetent recipients.

REASONS FOR THE CHOICE

Patients at risk of CMV infection are frequent in our Department of Transfusion Medicine, which operates within a large health care facility with Specialized Hospitals (Cardiology, Pediatrics) and specialist departments (Pediatric Blood Cancer, Cancer, Hematology, Infectious Diseases, Hepatobiliary Surgery for Transplant Recipients, Dialysis, Gynecology, etc.).

Moreover, the choice of using serological screening is due to the following reasons:

- availability of highly automated serovirological and molecular screening facilities, with interfacing between the automatic systems and the laboratory computer system (LIS);
- presence of qualified staff;
- low cost of immunoassays compared to the high cost of leucodepletion filters.

USEFULNESS OF THE SEROLOGICAL APPROACH

By IgG screening we identified the negative donors to use for recipients with high risk of infection, corresponding to approximately 30% of safe blood component units in our case. Moreover, IgM screening allowed us to identify the units from infected donors (confirmed by the CLIA method using LIAISON® XL - DiaSorin Saluggia – Italy), 118 with primary infection and 47 with reactivation or reinfection. As already commented, all of the donors were asymptomatic.

The percentage of IgM positives out the total was low (0.12%), but this in any case makes the approach useful as these units would have presented a very high risk of transmitting the CMV infection if transfused to patients at risk.

Although Italian law does not require the performance of the test for screening anti-CMV antibodies, we think it is in any case useful, in our context, to continue to support this type of approach, as it has three positive effects:

- the use of seronegative blood units for patients at risk;
- the identification of blood units with a high risk of transmitting the infection;
- the availability of new seronegative donors in particularly urgent cases.

REFERENCES

- Ho M.: Epidemiology of cmv infections. Reviews of Infectious Disease, 12, 1-10, 1990.
- Allain JP, Stramer SL, Carneiro-Proietti AB, Martins ML et al. Transfusion-transmitted infectious diseases. Epidemiol Inect 2009 Dec; 137 (12): 1776-80.
- Badami KG, McQuilkan-Bickerstaffe S, Wells JE, Parata M. Cytomegalovirus seroprevalence and 'cytomegalovirus-safe' seropositive blood donors. Transfusion 2009 Jan; 49(1): 134-45.
- Mazeron MC. Leukocyte depletion and infection by Cytomegalovirus. Transuf Clin Biol.2000 Jun; 7 Suppl 1:31s-35s.
- Thiele T, Kruger W, Zimmermann K et al. Transmission of cytomegalovirus (CMV) infection by leukoreduced blood products not tested for CMV antibodies: a single-center prospective study in high-risk patients undergoing allogeneic hematopoietic stem cell transplantation (CME). Transfusion 2013 Oct; 53(10): 2183-9.
- Ziemann M, Heuft HG, Frank K, Kraas S, Gorg S, Henning H. Window period donations during primary cytomegalovirus infection and risk of transfusion-transmitted infections. Transfus Med Hemother. 2014 Feb; 41 (1): 40-4.
- Ziemann M, Henning H Prevention of Transfusion-Transmitted Cytomegalovirus Infections: which is the optimal strategy? Biol Blood Marrow Transplant 2013 Oct; 19 (10): 1480-92.
- Ziemann M, Juhl D, Gorg S, Henning H. The impact of donor cytomegalovirus DNA on transfusion strategies for at-risk patients. Biologicals, 2009 Apr; 37(2): 71-7.
- Natali A, Valcavi P, Medici MC, et al. Cytomegalovirus infection in an Italian population: antibody prevalence, virus excretion and maternal transmission. New Microbiol 1997; 20: 123-33.