Quarantine Plasma: Quo vadis?

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Keywords

HBsAg · HBV · HCV · HIV · NAT · Plasma · Quarantine · Transfusion-associated infections · Virus transmission · Quarantine period reduction

Summary

Upon the introduction of mandatory nucleic acid amplification technology (NAT) testing in Germany for HCV, quarantining of fresh frozen plasma (FFP) was reduced in 2002 from 6 to 4 months. In 2004 HIV-1 NAT and in 2005 anti-HBc testing were introduced to further reduce the residual transmission risks for transfusion relevant viruses. After testing more than 40 million donations by HCV NAT it became obvious that NAT testing has a very significant impact on viral blood safety by reducing the residual risk by a factor of 10. Only one documented HCV transmission occurred during more than 10 years of NAT testing in Germany, indicating that the remaining risk is marginal. Similar data were obtained for HIV-1. The question arises whether we could discontinue quarantining of FFP or further reduce the quarantining interval for retesting of the donor. This could facilitate logistics and reduce losses as quarantine FFP can be released earlier after donation and at regular donation intervals. Essential parameters for estimating the remaining infectious risks are the minimal infectious dose and replication kinetics of the viruses involved, the detection limits of the NAT tests applied, and the volume of plasma transfused. In essence it can be assumed that discontinuation of quarantining would only marginally increase the residual risk and that the reduction of the quarantine period to only 4 weeks would add an additional benefit to the viral safety of quarantine FFP.

Schlüsselwörter

HBsAg · HBV · HCV · HIV · NAT · Plasma · Quarantäne · Transfusionsassoziierte Infektionen · Virustransmission · Quarantänezeitverkürzung

Zusammenfassung

In Deutschland wurde 2002 die Quarantänelagerung von therapeutischem Frischplasma (FFP) als Reaktion auf die seit 1999 vorgeschriebene HCV-Nukleinsäureamplifikationstechnik (NAT)-Testung von 6 auf 4 Monate reduziert. 2004 wurde die HIV-1-NAT und in 2005 der Anti-HBc-Test eingeführt, um das Restrisiko durch transfusionsrelevante Viren weiter zu senken. Die Testung von über 40 Millionen Spenden in Deutschland mit der HCV-NAT über mehr als 10 Jahre ergab nur noch einen Übertragungsfall. Dies resultiert in einer zehnfachen Reduktion des Restrisikos durch die NAT-Testung. Ähnliche Daten liegen für HIV-1 vor. Es stellt sich nun die Frage, ob die Quarantänelagerung von FFP überhaupt noch sinnvoll ist oder ob sie ohne Sicherheitseinbußen verkürzt werden könnte. Die entscheidenden Parameter, um das verbleibende Restrisiko zu bestimmen, sind die minimale infektiöse Dosis und die Replikationskinetik der betroffenen Viren sowie die Nachweisgrenze der angewandten NAT-Tests und das transfundierte Volumen. Als Fazit ergeben sich ein nur geringfügig erhöhtes Restrisiko, wenn die Quarantänelagerung beendet würde, und ein leichter Zugewinn an Sicherheit, wenn die Quarantänelagerung auf einen Zeitraum von zirka 4 Wochen verkürzt würde.

Introduction

Therapeutic plasma may not be as affected as red cells and platelets by the anticipated absolute and relative shortage of blood components in the near future. However, any losses of therapeutic equivalents should be kept as low as possible. At present, all three types of therapeutic plasma (quarantine fresh frozen plasma (QFFP), pathogen-reduced single-donor plasma, solvent/detergent-treated plasma) suffer from losses – in quantity and/or activity – during their manufacturing processes.

QFFP can be released from quarantine only when the donor returns to the blood center and tests repeatedly negative for the transfusion-relevant viruses HCV, HIV-1/-2 and HBV. This measure takes into account that a recently infected donor can be viremic without testing positive in serological antibody/antigen screening assays (diagnostic window). With the given sensitivity of serological tests, most donors will seroconvert from negative to positive within 6 months after infection. Therefore, quarantining for 6 months of fresh frozen plasma (FFP) has been introduced as a convenient and affordable additional safety measure for a blood product that is stable at low temperatures for up to 3 years. Depending on the local logistics and donor population (repeat vs. single donors, inter-donation intervals), varying and significant numbers of QFFP units cannot be released from quarantine and may be lost or converted to fractionation plasma if donors do not return for repeated testing in time.

Alternative methods to increase the viral safety of therapeutic plasma comprise pathogen reduction of single donor plasma or solvent/detergent treatment of pooled plasma. These methods allow the release of plasma without quarantining over a long period of time, and donors need not to be invited again for retesting. However these methods are very expensive and suffer from losses through reduced therapeutic activity due to the inactivation process. Like QFFP, pathogen-reduced plasma still has a residual infectious risk although risk reduction is more efficient and comprises an extended range of pathogens that are affected.

During the past decade not only pathogen reduction measures have improved significantly. Serological assays were developed that combine antibody and antigen detection in the same assay format, so-called combo assays, which reduced the diagnostic window significantly [1–3]. Moreover, the nucleic acid amplification technology (NAT) emerged and has been introduced into routine donor screening, thus further reducing the diagnostic window by its superior sensitivity [4]. The latter has already provoked a reduction of the quarantine period for QFFP in Germany from 6 to 4 months without any loss of viral safety [5]. After more than 10 years of NAT testing in Germany the residual risk for HCV and HIV-1 transmission was reduced roughly by one order of magnitude. In addition many blood centers have introduced combo assays as routine serological screening tests. Therefore, our quarantining policy deserves to be reconsidered.

Risks and Benefits of Reducing the Quarantine Storage Time

German blood centers were the first worldwide to voluntarily introduce NAT testing for all blood components on a daily routine basis [4]. This was preceded by NAT testing of plasma since storage time and quarantining facilitated the logistics of this challenging testing procedure in the early days. In 1999 HCV NAT became mandatory as in many other countries [6]. Although voluntary testing in big centers encompassed also HIV-1 and HBV NAT, it took up to 2004 until HIV-1 NAT was mandated [7]. A consequence of NAT testing in Germany was not only the reduction of the quarantine period of FFP from 6 to 4 months in 2003 but also the discontinuation of ALT testing in 2004 [8].

As a convenient and affordable alternative to increase blood safety with respect to HBV transmission, primarily by chronically infected occult HBV carriers, anti-HBc testing was introduced in 2006 [9]. HBV NAT is still performed as a voluntary test by most German blood centers. Main reasons for not mandating HBV NAT so far are the insufficient sensitivity in mini pools as the general approach for NAT testing in Germany and the tremendous financial and logistical efforts if more sensitive methods like single donation testing would be applied.

It is generally anticipated that further reduction of the quarantine storage time may lead to an increased residual risk of viral contamination of QFFP. This holds true even more for the discontinuation of quarantining. For a detailed estimate the residual risk has to be considered for each contaminating virus individually.

HCV

HCV was the main reason for the initial 6 months of quarantining of FFP. Antibodies against the virus appear late in circulation after an infection, leaving a diagnostic window phase of 2–4 months on average [5]. Most infected individuals seroconvert within 6 months after infection, and in rare cases this period may last even longer [5]. This window period not only depends on the immunological response of the infected individual but also on the sensitivity of the antibody screening assay applied. For decades the development of more sensitive assays was hampered by the HCV patent which did not allow diagnostic companies to establish assays without antigen preparations from the patent holder. Because of lacking competition, sensitivity of anti-HCV antibody assays improved only slowly.

The recently introduced HCV core antigen (and combo) assays reduced the diagnostic window by detecting the virus instead of detecting antibodies produced against the virus. Detection limits achievable with such formats are close to that of HCV NAT tests [10, 11].

Almost all diagnostic companies active in the blood screening business provide combo assays to a similar price as pure antibody or antigen assays. However, they are not as sensitive

as NAT tests, and therefore it is reasonable to calculate residual risk for HCV via the detection limits of NAT tests only. The mandated detection limit for HCV NAT tests was set by the Paul-Ehrlich Institute (PEI) to 5,000 IU/ml individual donor plasma independent from pooling and the pool size. Although pool sizes differ in Germany in a wide range, almost all of them exceed the required detection limit by a factor of 5–10. Therefore, detection limits of 1,000 IU/ml single donor plasma can be taken as worst case scenario.

There are different approaches to estimate and calculate the residual risk for transmitting viruses by transfusion of blood components which lie beyond the scope of this article. However, some basic ideas and estimates are applicable. The diagnostic window for HCV antibody tests was calculated to last about 60–80 days [12]. This quite long period contrasts the rapid increase (ramp-up) of viral load in the blood after an infection and the high viral titer in the following plateau during the antibody negative phase. However, this specific behavior made HCV NAT the most efficient measure in reducing the residual infectious risk in transfusion medicine. HCV doubles in the ramp-up phase within 0.45 days [13]. The ramp-up phase is preceded by a pre-ramp-up phase with very low titers, and the virus is virtually absent from the peripheral blood immediately after infection during the eclipse phase which is preceding the pre-ramp-up phase. So-called ‘blips’ were described during which little amounts of viral RNA may be detected even in this earliest eclipse phase. However, infectivity of ‘blip’ plasma could never be shown similar to eclipse phase plasma without ‘blips’ [13, 14]. This contrasts the following pre-ramp-up and ramp-up phases in which the virus exerts its highest infectivity [13]. Only 1–10 viral particles are most probably sufficient to initiate infection when transfused by a blood component. In later stages when antibodies are present infectivity is significantly reduced, and usually more than 100 viral particles are needed to be transfused to transmit the virus.

Red cell concentrates with about 20 ml residual plasma would be detected by present pool HCV NAT tests with 95% likelihood when it contains approximately 20,000 viral particles (given a 95% detection limit of 1,000 IU/ml). The 50% detection limits, however, are much lower and go down to below 500 IU/ml, converting to less than 10,000 particles per unit. Considering the doubling time of 0.45 days for HCV, we can calculate the remaining infectious window during which a donor is infectious and cannot be detected by our present pool NAT tests [13]. Starting with one copy per 20 ml it would last about 6 days until 10,000–20,000 IU would be reached. All numbers are approximate and worst case scenarios which are not based on exact calculations. With FFP that contains little more than 200 ml plasma per unit we would transfuse approximately 10 times more infectious particles resulting in a higher residual risk and a slightly longer infectious window of about 7.5 days. Given on average 6 months as donation interval for repeat whole blood donors in Germany, this will not increase significantly the residual risk for whole blood-derived FFP.

However, if we look at plasmapheresis donors who are allowed to donate plasma every second day, it could happen that such donors donate 3 times an infectious unit within their infectious window of 7.5 days without being detected by our present HCV NAT tests. For that reason, quarantining cannot be discontinued for such donors as far as sensitivity of NAT testing cannot be increased by more than two orders of magnitude. For whole blood-derived FFP it would be sufficient if the sensitivity would be increased by one order of magnitude to reach a similar residual risk as for red cells and platelets. However, the relatively higher infectious risk of FFP compared to the other components would persist, although at a much lower level.

Besides theoretical considerations, the residual risk can also be estimated with the help of clinical data. Only one breakthrough transmission occurred in Germany through red cell concentrates after more than 10 years of mandated NAT testing [15]. No transmission was due to platelets and QFFP. During this time more than 40 million red cell concentrates had been transfused, indicating a very low residual risk. Given the previous theoretical considerations we can estimate a similar or only marginally higher risk for whole blood-derived FFP. However, with respect to apheresis FFP, discontinuation of quarantining would not be justified unless there would be new advancements that improve significantly the sensitivity of HCV NAT.

Therefore we need to discuss the possibility of further reducing the length of quarantining which may be beneficial for logistics and economics and would reduce losses of QFFP. From the NAT point of view the reduction of quarantining from 6 to 4 months was a compromise that primarily reduced losses in such kind of transfusion services to which donors return only every 6 months on average. Given the maximum frequency of whole blood donations per year, it would have been more efficient to reduce the quarantine period to 3 months or less. There would be no additional viral risk since the average HCV seroconversion window is 60–80 days and HCV NAT has proven its high efficiency and reliability.

Could the quarantine period be reduced further? HCV replicates rapidly and at a very high rate, resulting in a steep ramp-up phase and a stable high titer viremia until antibodies become reliably detectable. Therefore it would be reasonable to reduce the quarantining period to a little more than 1 week, considering the above calculation of the time until 1 infectious viral particle per QFFP unit (0.005 IU/ml) has replicated to a level detectable by HCV NAT in pool testing (500–1,000 IU/ml). In practice, however, it may be advisable to reduce quarantine storage to 2–4 weeks for providing some safety margin.

HIV-1

The HIV-1 pre-seroconversion window period is shorter than that for HCV and may last between 2 and 5 weeks [13]. It was therefore never the determining factor for the length of quarantining of FFP. In some countries HIV-1 p24 Ag testing had

been introduced which reduced the window further by some days. Although HIV-1 p24 Ag testing had never been implemented in Germany, HIV-1 NAT was mandated as an alternative measure years after the HCV NAT [7]. Main reasons were the lacking reliability and the required sensitivity of HIV-1 NAT to significantly reduce the residual infectious risk. Present HIV-1 pool NAT procedures reach similar or little higher detection limits as the HCV NAT. In combination with the lower doubling rate and lower viremia titers it reduces not only the analytical but also the clinical sensitivity of the HIV-1 NAT compared to HCV NAT. However, the basic assumptions for HCV are valid for HIV-1 as well. It is anticipated that 1 viral copy may be infectious if transfused, although it has been reported that the minimal infectious dose of antibody-negative plasma may in some cases lie above 100 viral particles [13]. Several reasons were discussed. Upon storage at low degrees of centigrade for an extended period of time HIV-1 may be altered and lose infectivity. Host immunity and other host factors like genetic make-up may also apply. With respect to FFP, reduction of infectivity may occur due to freeze-thawing; however, deep freezing of virus in the presence of plasma proteins stabilizes HIV-1 and other viruses and is used for long-term storage in other settings. It can be assumed that similar to HCV one viral particle will be infectious if transfused, in particular if the plasma is collected from a donor in the viral pre-ramp-up and ramp-up phase. Viruses from donors who have already developed antibodies are less infectious, and the minimal infectious dose may be more than one order of magnitude higher.

Taking into account the HIV-1 doubling time of 0.85 days [13] and a similar detection limit of HIV-1 pool NAT, the infectious window can be calculated to last 12 days for FFP. This would still match with the proposed quarantine period of 2–4 weeks, although 2 weeks seem to be a borderline safety measure.

HIV-1 elite controllers pose a specific risk to the blood supply. About 2–4% of HIV-1-infected persons are able to control HIV-1 replication over an extended period of time. They test anti-HIV-1-positive and NAT-negative in most screening tests [16]. However, viral nucleic acids can be detected in peripheral blood at a very low concentration. It can only be estimated that there will be a similar diagnostic window as for ‘regular’ infections, resulting in a risk that cannot be reduced by NAT testing. Quarantining will most probably reduce this risk but the optimal interval is hard to estimate. Since it happens rarely and such persons belong to risk groups to be excluded from blood donation, the overall risk should be minimal and negligible as a specific risk for FFP.

HBV

HBV and HBV diagnostics are quite different compared to HCV and HIV-1. The serological HBsAg screening assay is a direct test that detects HBV surface proteins rather than anti-HBV antibodies. HBV replicates at high load of up to 10^9 –

10^{11} /ml and sheds from liver cells HBsAg into the blood stream at concentrations of up to 10^{14} /ml. This enabled diagnostic companies from the very beginning to develop relatively sensitive direct HBsAg screening tests. However, HBsAg usually disappears very rapidly after an acute resolving infection, and therefore HBsAg tests cannot contribute to reducing the infectious risk by retesting the donor after quarantining for 6 months. Anti-HBc antibodies which persist life-long were not reliably accessible for blood screening because anti-HBc tests suffered from a high rate of false-positives and false-negatives until recently. The introduction of mandatory anti-HBc testing in 2005 had only an indirect effect on the safety of QFFP because anti-HBc antibodies appear late (months) after infection. In some cases they appear later than anti-HBs antibodies when the virus has been cleared from the blood stream or viral load has fallen far below the detection limit of very sensitive NAT tests. Only chronic occult HBV infections (OBI) can be detected which are characterized by a positive anti-HBc test and negative HBsAg test. Anti-HBs and HBV NAT may be positive or negative [17].

For reducing the diagnostic window phase of HBsAg tests, very sensitive NAT tests must be applied. Only by enrichment steps through centrifugation of diagnostic mini pools, pool NAT can be sensitive enough to generate an additional risk reduction compared to HBsAg testing. Others favor individual donation (ID)-NAT testing approaches with high input sample volumes to increase sensitivity of HBV NAT [18]. In practice, we can assume that present pool NAT methods which are applied in Germany and utilize virus enrichment procedures may be about as sensitive as or, even more sensitive than, HIV-1 or HCV NAT tests. With such detection limits it has been shown that the diagnostic window can be reduced by some more days, depending on the sensitivity of the HBsAg test applied.

Among those discussed here, HBV is the virus with the longest doubling time of 2.6 days [13]. The minimal infectious dose is similar to HIV-1 and HCV and must be estimated to go down to one viral particle that must be present in a blood component. Considering a detection limit between 100 and 200 IU/ml which converts to 500–1,000 viral copies (particles) per ml and the volume of >200 ml FFP, the infectious window can be calculated to extend to 36 days. This corresponds to the infectious window for red cell concentrates if no NAT would be applied, whereas ID-NAT would still leave 21 days [13].

These calculations imply that 4 weeks quarantining would not be sufficient to reduce the HBV infectious risk for QFFP. However, a 4-month period may be even worse since not only the HBsAg titer but also the concentration of viral nucleic acids in plasma will probably fall below the detection limits of the respective tests during this period of quarantining. Thus, shorter quarantining will be better with respect to HBsAg and HBV NAT detection of contaminating virus upon retesting. Moreover, a significant number of HBV infections run without symptoms and detectable HBsAg and can be termed as

acute occult HBV infections. Therefore, even no quarantining would most probably not increase the residual risk for FFP since the present period of 4 months is not efficient for HBV. The HBV safety of QFFP relies solely on the initial HBsAg, anti-HBc and NAT screening, similar to red cell and platelet concentrates.

Conclusion

Due to viral replication kinetics and present detection limits of NAT tests, quarantining of FFP cannot be discontinued without some little increase in residual risk for the transmis-

sion of HCV and HIV-1 which is hard to quantify exactly. HBV transmission safety would most probably be not affected. Thus, quarantining can, after the introduction of NAT testing, only marginally increase viral safety and only if it would be reduced to about 1 month which is favorable primarily for HBV detection in QFFP from pre-seroconverters. With increasing sensitivities of NAT tests the favorable effect on viral safety of quarantining of FFP will decline further.

Disclosure

The author declared no conflict of interest.

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