The lack of transfusion-transmitted syphilis in the United States in the past 30 years has led us to question the rationale for continued syphilis testing of blood donors. In addition, the significance of a confirmed positive syphilis test result in a blood donor is not clear. The following 2 questions have been raised: (1) Are we detecting any truly infectious donors? and (2) If not, what is the significance of a confirmed positive test for syphilis in contemporary blood donors? This review will summarize what is and what is not known about syphilis testing in blood donors and will discuss the need for further research to answer these questions.

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SYPHILIS is an infectious disease caused by the spirochete T pallidum. This association was first described in 1905, with the demonstration of spirochetes in Giemsa-stained fluid from syphilitic lesions.1 Person-to-person transmission can occur during sexual intercourse, congenitally, or rarely, through other means, including accidental direct inoculation by a blood transfusion.2

Early in the 20th century, syphilis was a major public health problem. Transfusion-transmitted syphilis was first described in 1915. By 1941, 138 cases had been described in the literature.3 Serologic tests for syphilis (STS) have been performed on blood donations for more than 60 years, which is the longest of any infectious disease test. Transfusion-transmitted syphilis has become nonexistent in the United States because there have been no reported cases in more than 30 years. Yet, of all the tests performed on blood donors, least is known about the current rationale for this test and the significance of the test results in the blood-banking environment. There are 2 questions that we keep asking: (1) Are we detecting any truly infectious donors? and (2) If not, what is the significance of a confirmed positive test for syphilis in the contemporary allogeneic blood donor?

This review will summarize what is and what is not known about syphilis testing in blood donors and will suggest further work, the results of which may help answer these questions and thus the potential to change our testing practices or how we manage the counseling of blood donors who have positive STS.

WHAT WE KNOW

Natural History of Disease

In nature, syphilis is contracted during sexual intercourse by passage from genital lesions of 1 partner though the mucous membranes of the other partner. Intracutaneous inoculation results in the organism multiplying at the primary site and presence of organisms in local lymph nodes within minutes and systemic dissemination within hours.1,5 Within 4 days, the spirochetes disseminate by invasion of the local lymph nodes causing swelling (lymphadenopathy) followed by seeding to the bloodstream in large numbers.5 Localized lymphadenopathy and primary lesions (chancres) develop in 9 to 90 days, with an average of 21 days.6,7 The primary chancre can be found in up to 97% of infected individuals, although up to 60% may not remember having had a lesion. Chance healing will occur spontaneously in about 2 to 6 weeks, although lymphadenopathy persists.4,7 Progression from primary to secondary disease occurs in approximately 46 days (range 30 - 70 days).7

If untreated, the spirochete begins migrating to the bloodstream, and the disease progresses to the secondary phase 6 to 12 weeks after exposure (2 - 8 weeks after development of the chancre),5,7 with a corresponding vigorous antibody response.5 Once the organism has invaded the bloodstream, it continues to infiltrate all organs and body fluids.8 Peak spirochtemia with high organism load and systemic infection occur during secondary syphilis. A generalized condition with parenchymal, systemic, and mucocutaneous manifestations occurs.7 Secondary phase is the most clinically apparent and is characterized by acute infection caused by...
multiplication and systemic dissemination of the organism. During secondary syphilis, spirochetes are cleared from the bloodstream, probably by a lytic process involving complement. Untreated secondary disease lasts a mean of 3.6 months, with a range of 1 to 12 months. In 50% of cases, the patient will no longer be infectious or exhibit any symptomatology and will be considered cured. The other 50% will go on to a latent phase. This stage progresses to the asymptomatic latent phase in which a positive serologic test for syphilis will be the only indicator of infection.

The latent stage of disease is characterized by complete asymptomatology with positive serology being the only evidence of infection. During this time, the organisms remain dormant and are undetected in blood and tissues. There are no cutaneous manifestations or clinical symptoms, but the organism may be actively replicating in target organs. Although most patients in late latent syphilis remain asymptomatic, one third go on to tertiary syphilis 10 to 20 years later. This latent stage may persist for life or progress several years or decades later to tertiary syphilis, which may result in gummatous lesions, cardiovascular, or neurologic disorders. Approximately 30% of untreated patients progress, over a period of many years, to the tertiary stage with progressive disease occurring in the ascending aorta and central nervous system.

Antibody Development and Testing

In 1906, August von Wasserman developed the first test for syphilis and so began serologic testing for syphilis. This test was a nontreponemal serum reagin test, the availability of which allowed for the testing of blood donors beginning in the 1940s. Over the years, tests have been modified and changed, improvements made in specificity, and treponeme-specific antibody tests developed. More recently, treponemal-specific antibody tests have been automated. A summary of these tests is described in Table 1.

<table>
<thead>
<tr>
<th>Test</th>
<th>RPR</th>
<th>FTA-ABS</th>
<th>PK™ TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methodology</td>
<td>Charcoal flocculation</td>
<td>Immunofluorescence</td>
<td>Hemagglutination</td>
</tr>
<tr>
<td>Becomes positive</td>
<td>~ 2 weeks post-exposure</td>
<td>~ 2 weeks post-exposure</td>
<td>~ 2 weeks post-exposure</td>
</tr>
<tr>
<td>Remains positive</td>
<td>3 months - 1 year</td>
<td>Probably for life</td>
<td>Probably for life</td>
</tr>
<tr>
<td>Treated</td>
<td>Decades; decreasing with latency</td>
<td>Probably for life</td>
<td>Probably for life</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>86% (77-100)</td>
<td>76% (69-90)</td>
<td></td>
</tr>
<tr>
<td>Primary phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary phase</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Latent</td>
<td>98% (95-100)</td>
<td>97% (97-100)</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>98% (93-99)</td>
<td>99% (98-100)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Antibody Detection by Test in Untreated Syphilis
Treponemal tests. Because of the nonspecific nature of the tests, further research led to the development of treponemal specific tests. The latter are designed to detect antibodies directed against treponemal cellular antigens. The fluorescent treponemal antibody absorption test (FTA-ABS) is the most commonly used for confirmation of a positive STS. This test has been available since the mid 1960s and uses the pathogenic Nichols strain of *T. pallidum* as the antigen. The test detects 2 different antibodies; the first, called a group antibody, reacts with antigens shared with other treponemes. This antibody is present in low titres in most normal nonsyphilitic sera and may be a biological balance between natural and immune antibody, or it may be produced after exposure to other commensal treponemes in the body. Removal of the group antibody by absorption allows for the detection of a second *T. pallidum*-specific antibody. Both antibodies are found in patients with *T. pallidum* infection, and the group antibody actually predominates in all phases except secondary syphilis. The addition of the fluorescein tag results in fluorescent *T. pallidum*, and sample fluorescence is measured against a standard control.

As a manual test, 1 downfall of this test is the subjective evaluation required by the observer. Because the FTA-ABS test is manually performed, there is a certain amount of misclassification because of the subjective nature of result interpretation. (This is also true for RPR.) In the blood donor setting, the practice is often to interpret the results erring on the side of caution, thus increasing the number of reactive (RPR) and positive (FTA-ABS) test reports and thereby increasing disease misclassification. Borderline or equivocal reading have less than a 5% chance of being associated with syphilis.

In 1965, hemagglutination assay technology was applied for the development of treponeme-specific testing. The resulting *T. pallidum* hemagglutination assay (TPHA) was further modified to a microhemagglutination (MHA-TP) format. This test could be used qualitatively, quantitatively, and was simple to use. Recently, the MHA-TP technology has been automated (PKXMTP, Olympus, Irving Texas), eliminating the subjectivity associated with the manual test interpretation. TPHA tests have detectable reactivity approximately 4 weeks after exposure. Antibody titers increase dramatically during secondary syphilis. During latent syphilis, or after treatment, the titers decline and may become undetectable, although even after successful treatment, most infected individuals test positive for life. Because nonpathogenic treponemes cannot be immunologically distinguished from *T. pallidum*, the TPHA tests will also detect antibodies to these organisms.

Agreement between the TPHA and FTA-ABS tests vary from 87% to 93%, depending on the study population. It has been suggested that the MHA-TP test may not be appropriate for routine use as screening procedures because 1% of the general population will have false-positive results. However, current use of the PKXTP for syphilis testing is standard in the blood-banking environment because of the ease of automation.

Sensitivity of all STS varies with an increase seen as time from exposure increases. There is conflict in literature reviews regarding the earliest positive results for syphilis testing when comparing nonspecific cardiolipin tests with the treponeme specific tests. However, studies specifically evaluating this issue suggest that treponeme-specific antibody detection occurs at approximately the same time as the nonspecific and possibly slightly sooner (Table 1).

Other Research Tests

Rabbit infectivity testing. The “gold standard” for syphilis infectivity is rabbit infectivity testing (RIT) because it is a direct measure of infectivity. It appears that RIT requires approximately 10 viable organisms per inoculum to transmit infection. Intertesticular or interdermal inoculation causes formation of a localized tissue lesion that remains infective for life, may be transferred to another animal, and causes positive STS. Incubation may require up to 90 days and multiple animal passages. Because it is impractical and expensive, it is used only in research settings and as a sensitivity reference for PCR testing.

Western blot. Western immunoblot technology is used in the blood donor environment to perform confirmatory testing of retroviruses. Extension of this technology has led to the development of a test using whole-cell, *T. pallidum* antigen. Detection of at least 3 or 4 major antigens having molecular masses of 15.5, 17, 44.5, and 47 Kd indicate a positive test. With clinically confirmed samples, the sensitivity of the assay is 93.8%, and the specificity is 100%. Agreement between the
Western blot (WB) assay and the FTA-ABS is greater than 95%, with the WB having equal or greater sensitivity. The WB assay showed no false positive or equivocal reactivities for nontreponemal specimens from individuals with previously false-positive test results, elevated gamma globulin, or antinuclear antibody, but 1 false-positive test results in a normal blood donor. Unfortunately, the number of samples tested in each group was small (19, 10, and 15, respectively). Restriction of the result interpretation to exclude bands of molecular mass between 30 and 40 Kd minimizes cross-reactivity with flagellar and nonspecific proteins of other treponemes.\(^{17}\) Testing by WB allows for the detection of both IgM and IgG antibodies, with detection of IgM antibody suggesting recent or active infection.

**Polymerase chain reaction.** Polymerase chain reaction (PCR) has been used extensively for viruses and currently is in use for the detection of hepatitis C virus and human immunodeficiency virus (HIV) in blood donors. The principle of PCR technology is the use of successive rounds of in vitro replication of nucleic acid sequences using primers complementary to specific targets. Millions of copies of the target gene are made, making it possible to detect minute quantities of a transmissible agent in the blood. Although this technology is being used with transfusion-transmitted viruses, little work has been done related to transfusion-transmitted bacteria (ie, *T. pallidum*).\(^{18}\)

**DNA.** The first description of PCR for testing of *T. pallidum* DNA was in 1990. This technique amplified the TmpA and 4D genes, had a sensitivity to approximately 65 organisms per 0.5 mL, and a specificity of approximately 96%.\(^{16}\) In 1991, the description of the use of the highly conserved gene for the *T. pallidum* 47 Kd immunogenetic membrane protein was first reported.\(^{19}\) This target is commonly used in PCR technology because it is rarely found to cross-react with other commensal spirochetes.\(^{8}\) Early studies using normal sera and cerebrospinal fluid from 9 adults with untreated syphilis reported a sensitivity of 100% in samples with at least 10 organisms, but inhibitory effects caused a lower sensitivity at lower organism concentrations. Specificity was 100% against other bacterial flora and human DNA.\(^{19}\)

More recently, the 47 Kd target has been used in a multiplex test format that includes testing for *T. pallidum* DNA PCR with known infected samples compared with dark-field microscopy with a reported sensitivity of 91% and specificity of 100%. Analysis of unrelated organisms and other commensal treponemes indicated no cross-reactivity.\(^{8}\) When compared with individual confirmatory PCR assays, concordance was 100%. When comparing PCR with STS, the specificity discrepancy (PCR negative, STS positive) between the 2 tests was attributable to a possible sampling error or a lower specificity of the dark-field microscopy because of commensal organisms and/or serologic false positives. The sensitivity discrepancies (PCR positive, STS negative) were reported to potentially occur in patients with early syphilis when STS specificity may be as low as 75%.\(^{20}\)

One limitation of PCR testing for *T. pallidum* is the inability to differentiate between the DNA of viable or dead organisms.\(^{8}\) Persistence of the *T. pallidum* DNA in successfully treated individuals with neurosyphilis may be because of the very stable nature of the *T. pallidum* DNA biopolymer. Therefore, the length of time that there is persistence of dead organism is unknown.\(^{21}\)

**RNA.** New tests based on the amplification of *T. pallidum* RNA have been developed. Although 2 copies of ribosomal DNA have been identified in *T. pallidum*, the number of copies of ribosomal RNA per cell is not known, although it is assumed that like other bacterial species there are many copies. No cross-reactivity with other spirochetes has been shown, including testing with *B. burgdorferi* and the nonpathogenic spirochetes. Because it is unknown if DNA detection is derived from viable or dead organisms, it is possible that RNA detection suggests the presence of living versus dead organisms because RNA is rapidly degraded after organism death occurs, making it a more reliable indicator of potential infectivity.\(^{22}\) Reverse transcriptase PCR (RT-PCR) is a powerful tool for detection of rare RNA and is more sensitive than DNA PCR because multiple copies of RNA are found per organism, allowing for the detection of less than 1 genome equivalent.\(^{23}\)

Current RNA RT-PCR methodology uses the 16S ribosomal RNA of *T. pallidum* as a template with the production of complementary DNA and has used amniotic fluid, sera, and cerebrospinal fluid samples. Samples spiked with *T. pallidum* organisms have shown sensitivity to detect as low as \(10^{-2}\) to \(10^{-3}\) *T. pallidum* equivalents of organisms (that is, 10\(^2\) or 10\(^3\) RNA copies per organism
compared with 1 DNA). This means that a sample can be diluted over 100-fold more than for DNA-based PCR while still detecting organisms (provided there is at least 1 organism per sample). Sensitivity testing of 20 clinical isolates that had been infectious using RIT was 100%. No cross-amplification was detected by using other species as templates (100% specificity).

Recent Historic Perspectives of STS for Blood Donation

In 1985, although the requirement for syphilis testing was dropped from the American Association of Blood Banks Standards, the Food and Drug Administration (FDA) failed to support this change. The FDA's position at that time was that STS be retained as a potential surrogate for high-risk behavior for HIV infection, rather than as evidence of syphilis, and syphilis screening continued to be a mandatory requirement. In 1995, the National Institutes of Health convened a Consensus Development Conference, which included discussion of the extent to which tests for syphilis contributed to transfusion safety and whether their use as in current practice be continued or modified. The resulting recommendation was to continue syphilis screening of all blood donors until such time as more information was available regarding the effect of component storage conditions on *T. pallidum* survival and molecular techniques could assess the absence of *T. pallidum* in serologically positive donated blood. In 1999, the FDA requested the submission of available data to again re-evaluate the need for continued syphilis testing of blood donors. After presentation of data at the September 2000 Blood Product Advisory Committee Meeting, the committee voted that the current scientific data were insufficient to warrant discontinuation of donor testing for antibodies to syphilis.

Syphilis Screening as a Surrogate Marker for HIV

Although donor deferral strategies and routine testing for HIV have excluded nearly all HIV-positive donations, the usefulness of STS as a surrogate marker for HIV infection in seronegative blood donors is still being debated. In 1995, the National Institutes of Health also addressed the rationale of continuing syphilis screening as a surrogate marker for other transfusion-transmitted infections and concluded that the low efficacy was not sufficient by itself to warrant the continued use of syphilis screening in blood donors.

The most extensive evaluation of syphilis screening as a surrogate marker for HIV was published in 1997. The source population included 4,468,570 donations collected between 1992 and 1994 at 18 American Red Cross (ARC) sites. Of an estimated 13 HIV infectious window-period donations potentially donated during this time, 0.2 would have been removed because of a reactive STS alone at a cost of over $16 million. With the incorporation of HIV p24 antigen testing in 1995 and the addition of nucleic acid testing for HIV in 1999, the residual risk of HIV infectious window-period donations has been reduced even further, making the value of STS as a surrogate marker negligible, if of any value at all.

Routine Blood Donor Testing for Syphilis

Over the years, blood centers have shifted from the use of the nontreponemal tests (ie, RPR) to treponemal tests (ie, PK"T"M"T"P" hemagglutination) for donor screening. This has been primarily because of the ease of automation and the minimalization of technologist-dependent interpretation of test results. However, this has led also to detecting lifelong antibodies (versus antibodies suggesting current, recent, or persistent infection).

The current routine blood donor testing algorithm for syphilis includes the use of either a nontreponemal test (RPR) or an automated treponemal test (PK"T"M"T"P") as the screening test. If this test is reactive, it is confirmed by testing with an additional treponemal test, the FTA-ABS or an enzyme immunoassay. Sensitivity of the PK"T"M"T"P" test varies by phase of disease but has clinical sensitivity (that is, tested in individuals with clinically diagnosed disease) of 93.9% and specificity of 95.6%. The FTA-ABS has a relative sensitivity (that is, compared with clinical samples tested by PK"T"M"T"P") of 99% and specificity of 93%. Blood centers that use the PK"T"M"T"P" as a screening test may further test the donor using the RPR as a measure of recent exposure for the purposes of donor counseling.

It is important to point out that all of these tests have been developed for diagnostic use and not for use as screening tests. The use of diagnostic tests for screening low-risk populations results in a relative increase in false-positive test results, which
leads to a low-positive predictive value (PPV or percent of individuals who test positive and are currently infected) of a positive test. By using Baye’s theorem,25 the PPV of the combined tests (PKTMTP and FTA-ABS) can be calculated using the following formula:

$$PPV = \frac{\text{disease prevalence} \times \text{sensitivity}}{[\text{prevalence} \times \text{sensitivity}] + [(1 - \text{prevalence}) \times (1 - \text{specificity})]}.$$ 

Today, by using the incidence of clinical syphilis in the United States (2.6/100,000) as an estimate of prevalence, the PPV for disease detection in the general population can be calculated as being < 0.1%. Because blood donors are a low-risk population (and current methodology also detects antibody from old, treated disease), the PPV for this group may be even lower.

To show the number of donors in the United States per year affected by positive STS results, data from the ARC may be used and extrapolated. Between July 1998 and October 1999, approximately 7.45 million donations were tested for syphilis. Of these, 16,907 (~13,525 per year) had a reactive syphilis screening test result, and 40% had a confirmed positive FTA-ABS (~5,400 per year). Of the confirmed positives, 27% were RPR reactive. Because the ARC draws approximately 50% of US blood donors, doubling of these figures would yield an estimate of nationwide figures.

Interpretation of Positive Syphilis Test Results in Blood Donors

It is unclear if our multiple testing algorithms are truly identifying any infectious donations. A cases series report of platelet concentrates and serum samples from blood donors with PKTMTP+, FTA-ABS+ STS, tested for T. pallidum DNA and RNA was presented at the September 2000 Blood Products Advisory Committee meeting in Bethesda, MD. These samples were tested using the DNA test that was T. pallidum pol A–gene specific, a DNA test that was a multiplex PCR using a T. pallidum 47 Kd gene target and/or an RT-PCR test for RNA using T. pallidum 16S ribosomal RNA as a template for production of a complementary DNA target, as previously described. At least 100 samples (50 RPR+ and 50 RPR−) were tested by each method. All samples were negative for evidence of T. pallidum DNA or RNA. The lack of demonstrable T. pallidum DNA or RNA suggests that the probability that the blood of donors who have confirmed positive syphilis test results is infectious for syphilis is 0% to 3%.26 These results are in contrast to recent data reported from the analysis of 2 syphilis outbreaks in the United States, which found that T. pallidum DNA could be detected in the blood of untreated, infected individuals during all phases of syphilis infection.27,28 By extension, it is unlikely that blood donors with confirmed positive syphilis tests are truly infected with syphilis.

To what can we attribute confirmed and unconfirmed positive syphilis test results in blood donors? There is ample literature addressing conditions associated with false-positive syphilis test results.6,7,8,12,29,30,31 To determine if these conditions applied to blood donors, a history of conditions reported to be associated with false-positive syphilis tests (Table 2), and/or a previous history of syphilis infection was assessed by a case-control study of donors with reactive (both confirmed and nonconfirmed) and nonreactive PKTMTP test results by using an anonymous mail survey. Among responding blood donors, approximately 50% of donors with FTA-ABS–positive test results reported a syphilis history. There was no difference in reported false-positive conditions for either FTA-ABS–positive or FTA-ABS–negative cases, compared with controls. These results suggest that (1) a large proportion of confirmed positive test results are a result of old, treated infection and (2) although historically conditions in the literature associated with false-positive test results have been used to explain both unconfirmed and confirmed

<table>
<thead>
<tr>
<th>Table 2. Published Causes of a False Positive STS</th>
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<tbody>
<tr>
<td>Non-specific Tests</td>
</tr>
<tr>
<td>Pregnancy</td>
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<tr>
<td>Some viral/bacterial infections</td>
</tr>
<tr>
<td>Liver disease/hepatitis</td>
</tr>
<tr>
<td>Vaccinations/immunizations</td>
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<td>Hypergammaglobulinemia</td>
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<tr>
<td>Leptospirosis</td>
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<td>Narcotic addiction</td>
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<td>Lupus erythematosus</td>
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<td>Rheumatoid arthritis</td>
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<td>Other autoimmune/collagen diseases</td>
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<td>Aging</td>
</tr>
<tr>
<td>Diabetes</td>
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<tr>
<td>Lyme disease</td>
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false-positive STS test results in healthy blood donors, this may not be appropriate.32

**Biological Properties Relevant to Potential Transfusion Transmission**

The properties necessary to classify an infectious agent as potentially transfusion transmissible include presence in blood; infectious asymptomatic, carrier or latent stages; and stability or growth of the agent in stored blood or blood components,33 such as red blood cells and platelet concentrates.

Several additional factors influence the probability of a blood donor being truly infected with syphilis and thus the probability of the presence of treponeme in blood. First, the frequency of syphilis infection in the general population has changed over time. In 1947, before the discovery and use of penicillin, the incidence of primary and secondary syphilis was 66.4 cases per 100,000 persons. The last syphilis epidemic was in 1990 when the incidence was 20.3 per 100,000 persons. More recently, the incidence of syphilis has decreased as a widespread disease (2.6/100,000 in 1998). This low incidence in the general population decreases the probability of a blood donor being infectious for syphilis. Second, properties of the different phases of syphilis infection may be related to the likelihood that an individual would present as a blood donor and/or that an individual's blood is infectious. Early primary syphilis is characterized by rapid dissemination that may precede antibody development and symptomatology and has resulted in documented transfusion acquired infection from blood donors in the seronegative window period. Although this potential has always been present, we still have not seen a documented case of transfusion-transmitted syphilis. Inoculation studies have shown that the infectious potential of whole blood stored at 4°C is directly proportional to the size of the inoculum.

Inoculum concentrations of $5.0 \times 10^4$ treponemes/mL, $1.25 \times 10^6$ treponemes/mL, and $2.5 \times 10^7$ treponemes/mL were able to induce syphilis in rabbits up to 36 hours, 72 hours, and 108 hours, respectively.35,36 Because current transfusion demands for red blood cells often lead to the transfusion of blood that is a few days old, it cannot be assumed that storage at 4°C would preclude transfusion transmission of the agent.10

**WHAT WE DO NOT KNOW**

Several hypotheses have been put forward to explain the lack of reports of transfusion-transmitted syphilis. These include: (1) a low incidence rate of the disease, (2) movement to all volunteer blood donors, (3) deferral of individuals with high-risk behaviour using donor history questionnaires, (4) serologic testing of blood donors, (5) the impact of refrigeration on the survival of the spirochete, (6) use of antibiotics in hospitalized patients, and (7) transmission with an as yet undetected infection.10

Preparation and storage of individual blood components may have an impact on the potential for transfusion transmission. However, several key factors that could affect this potential are unknown. First, the concentration of treponeme in the blood of a naturally infected individual at different phases of infection has not been published. Preliminary data on 5 blood samples from individual’s studied in a syphilis outbreak in Maricopa County, AZ, suggest that the concentration of treponeme varies from approximately 50 to 100,000 organisms per mL, depending on the phase of infection.7 Second, the early 4°C storage studies make no comparison to any other storage temperature. Therefore, no reliable information is available on the true effect 4°C storage has on *T pallidum* survival. Conversely, platelet concentrates are stored in bags designed to facilitate oxygen flow. This property of the platelet bag leads to an internal oxygen content (~15%) higher than that which can be withstood by the *T pallidum* spirochete (1% - 4%),37 although the actual impact on survival time is unknown.

Evidence that the *T pallidum* spirochete attaches to the surface membrane of phagocytes38 may have implications for the increased use of leukoreduction filters. Transfusion medicine is moving toward a policy of universal leukoreduction of all cellular blood components (i.e., red blood cells and platelets) for the removal of white blood cells. This
universal leukoreduction may impact the potential for transfusion-transmitted syphilis.

It is unlikely that a transfusion-transmitted case of syphilis would not be identified, based on past descriptions of transfusion-transmitted syphilis, which presented as acute, fulminant symptomatic secondary syphilis. In addition, very specific antibiotic use would have to occur to cure a patient with transfusion-transmitted syphilis. The acute symptomatology usually associated with the spirochetal phase of the disease may also make it unlikely that an infected individual would present as a blood donor. However, none of the hypotheses stated earlier have been fully tested.

Testing Limitations

A serious limitation of many published articles is the lack of a gold standard for diagnosis of syphilis in blood donors. The gold standard for syphilis testing, RTI, is not used routinely in the evaluation of licensed test kits for diagnosis and/or screening of blood donors. For the purposes of overall sensitivity determination, clinical diagnosis has been used as the gold standard. As new tests are developed, sensitivity and specificity testing are calculated and compared relative with the standard test in use at that time, rather than by testing large groups of samples from clinically diagnosed individuals. This type of comparison may be useful in comparing positive tests (ie, reactivity or titers) in individuals with independent assessment of disease but can be misleading when used with both negative and positive tests in populations without a gold standard disease classification. The resulting relative sensitivity and specificity values reported by the manufacturer may result in a high PPV in diagnosing populations likely to be infected but has questionable applicability when used for screening in low-risk populations, such as blood donors, and may result in disease misclassification.

It should also be noted that although reactive STS are confirmed with an additional test, the use of a multiple test algorithm with tests that lack independence of test results (have commonality of false positives or detection of old antibody) means that the additional test results may not provide additional diagnostic information. An assumption of increased specificity of a confirmatory test calculated using the results found when screen-reactive individuals are tested is misleading. This perceived increase in specificity is a result of a greater probability of disease after a second positive test result and reflects the increased prevalence in the population tested. As an example, for the FTA-ABS test, studies of unselected sera run in parallel do not support the increased specificity reported in the literature. In individuals who have no evidence of syphilis, even multiple positive tests are not a substitute for clinical judgment.

Information for Blood Donors

The relevance of a confirmed positive syphilis test result in an apparently healthy blood donor is an issue today. Although there is ample literature addressing false-positive test results associated with specific conditions when using the current methodology, these conditions may not be relevant to the contemporary interpretation of positive STS in blood donors.

For PKTMT confirmed donors, post-donation counseling information regarding the implication of a false-positive test result is necessarily incomplete as it is unknown what factors cause a false-positive test result in this population. For donors with FTA-ABS–confirmed results, a recent exposure to syphilis appears to be unlikely, and exposure to syphilis some time in the past appears to explain approximately 50% of FTA-ABS–confirmed positive test results. However, false-positive test results also occur without association to the conditions reported to cause false positives, for reasons which are currently unknown. It is known, however, that the majority (90%) of blood donors with confirmed positive syphilis test results continue to test confirmed positive on subsequent donations.

WHAT WE NEED

1. Follow-up of transfusion recipients for evidence of transfusion-transmitted syphilis would answer the question of whether or not this event ever occurs today. However, the broad scope and associated cost of this type of large-scale study of transfusion recipients would be prohibitive.

2. Follow-up of blood donors with confirmed positive syphilis tests for evidence of infection would answer the question of whether or not any infected blood donors present for donation. Again, the broad scope and associated cost of this type of study would also be prohibitive. However, collaboration with the
Public Health Service in interviewing individuals who represent subsequent to having a confirmed positive syphilis test at donation might be informative.

3. Specific studies on the compartmentalization of spirochetes in stored blood components and the effect of storage conditions and containers are needed to clarify what impact refrigeration (for red blood cells), oxygen tension (for platelets), or leukofiltration has on spirochete viability, and by extension, transmission potential. In addition, quantification of treponeme concentrations in the blood of naturally infected individuals is needed.

4. Manufacturers should provide sensitivity and specificity information resulting from the testing of samples from individuals with and without clinically diagnosed disease (as the gold standard) rather than relative sensitivity and specificity.

5. Because predictive accuracy varies with the clinical characteristics of the test population, a description of the predictive value in low-risk populations based on the prevalence of disease in that population should be described in the manufacturer's package inserts. This is especially important when a diagnostic test is used for screening a low-prevalence population, as is required in the blood banking environment.

6. With the characterization of the complete T pallidum genome, antigens and antibodies produced in response to these antigens, as well as the many test methodologies available (ie, PCR, Western blot), manufacturers should attempt to analyze and correct specificity problems. Alternatively, if donor spirochtemia is of primary concern, development of an inexpensive, sensitive, specific, automated test methodology for presence of spirochete (ie, PCR) is warranted.

7. Review of the current data on syphilis testing and the implications of both unconfirmed and confirmed positive STS in blood donors should be done to assure that predonation information and/or post-donation counseling of blood donors with positive STS is as accurate and complete as possible.

REFERENCES


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Genetically Modified Dendritic Cells in Cancer Therapy: Implications for Transfusion Medicine

Ronan Foley, Richard Tozer, and Yonghong Wan

Dendritic cells (DCs) are a heterogeneous population of antigen-presenting cells (APCs) identified in various tissues, including the skin (Langerhans cells), lymph nodes (interdigitating and follicular DCs), spleen, and thymus. Properties of DCs include the ability to (1) capture, process, and present foreign antigens; (2) migrate to lymphoid-rich tissue; and (3) stimulate innate and adaptive antigen-specific immune responses. Until recently, the ability to study DCs has been limited by their absence in most culture systems. It is now known that specific cytokines can be used to expand DCs to numbers sufficient for their in vitro evaluation and for their use in human immunotherapy trials. Human DCs can be derived from hematopoietic progenitors (CD34+-derived DCs) or from adherent peripheral blood monocytes (monocyte-derived DCs). Cultured DCs can be recognized by a typical veiled morphologic appearance and expression of surface markers that include major histocompatibility complex class II, CD86/B7.2, CD80/B7.1, CD83, and CD1a. DCs are susceptible to a variety of gene transfer protocols, which can be used to enhance biological function in vivo. Transduction of DCs with genes for defined tumor antigens results in sustained protein expression and presentation of multiple tumor peptides to host T cells. Alternatively, DCs may be transduced with genes for chemokines or immunomodulatory cytokines. Although the combination of ex vivo DC expansion and gene transfer is relatively new, preliminary studies suggest that injection of genetically modified autologous DCs may be capable of generating anti-tumor immune responses in patients with cancer. Preclinical animal studies showing potent antigen-specific tumor immunity after DC-based vaccination support this hypothesis and provide rationale to further evaluate this approach in patients. Preliminary human studies are now required to evaluate optimal DC dose, schedule of vaccination, route of delivery, and maturation of cultured cells. Initiation of these phase I/II cell therapy-based studies will occur in collaboration with hospital-based transplantation facilities. Issues relating to cell harvesting, storage, culture methodology, and administration will require the collaborative efforts of basic scientists, immunologists, clinical investigators, and transfusion medicine staff to ensure strict quality control of injected cellular products. This review is intended to provide a brief overview of clinical DC-based gene transfer.

ADMINISTRATION of high-dose cytotoxic therapy has shown efficacy in a selected proportion of patients with cancer. High-dose, chemotherapy/radiation-based treatments are now safely used in a variety of settings.1-3 Despite this advance, there remains a significant number of patients who fail to achieve a durable complete remission or who relapse after an initial favorable response. As a result, there is a need to evaluate alternative therapeutic strategies that may be used in combination with conventional cytotoxic-based therapies. Immunotherapy with cancer vaccines has attracted significant recent attention. Interest in immunotherapy is based on recent advances in our understanding of tumor immunology. These advances address concepts relating to (1) tumor-associated antigens (TAAs), (2) antigen presenting cells (APCs), and (3) effector cell subsets.

Dendritic cells (DCs) are specialized APCs capable of inducing a primary immune response.4-7 Autologous DCs can be expanded and genetically modified ex vivo.8-12 The technology to perform gene transfer to expanded DCs has led to the development of vaccination protocols that aim to enhance tumor antigen-specific immunity by introducing genes for tumor peptides or immunomodulatory cytokines into autologous DCs.13-16 Genetically modified autologous DCs are injected in an effort to stimulate an immune response against a specific protein(s) on target cancer cells. Generation of an anti-tumor immune response may then be able to prevent cancer recurrence (protective immunity) or induce regression of existing tumor(s) (therapeutic immunity). Although preliminary human studies suggest that DC-based vaccinations are both safe and feasible, questions remain regarding (1) the most effective dose and route of delivery, (2) the optimal state of DC maturation,