



Transition from Secondary Blood Test to Nucleic Acid Amplification for Safe Allograft Transplantation

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Background: Given the incubation period of viral diseases, a secondary blood test should be performed at least 3–6 months after the first test to ensure the safety of allogenic bone grafts obtained from living donors in some tissue banks. The allograft is discarded if a secondary blood test was unavailable. The secondary blood test can be replaced with a nucleic acid amplification test (NAT) to reduce the discarded allograft. The purpose of this study was to analyze the comparative efficiency of secondary blood test and NAT to determine the donor suitability of allogenic bone grafts.

Methods: Allogenic bones were retrieved from 452 living donors between January 2013 and December 2019. A secondary blood test was conducted in 182 patients and NAT was performed in 270 patients. The average age of donors was 69 years (range, 33–87 years). They included 86 men and 366 women. The initial blood tests including hepatitis B, hepatitis C, AIDS, and syphilis were conducted before retrieving grafts. The results were analyzed after the secondary blood test was performed at least 3 to 6 months after the first test because of the incubation period of the viral diseases. NAT was performed within 2 months after the first blood test.

Results: Sixty-seven of the 452 cases (14.8%) were discarded. In the secondary blood test group, 50 out of 182 cases (27.4%), and in the NAT group, 17 out of 270 cases (6.3%) were discarded. None of the 132 donors tested positive in the secondary blood test after testing negative in the first test.

Conclusions: It is extremely rare that the secondary blood test yields positive results in donors who tested negative in the initial test. However, quite a few grafts are discarded only because the secondary blood test is not available. In terms of economics and ethics, the secondary blood test may not be necessary or if required, a single test such as NAT for infectious diseases may be performed to determine donor suitability of allogenic bone.

Keywords: *Bone transplantation, Donor safety, Secondary blood test, Nucleic acid amplification techniques*

Human tissue includes bone, cartilage, fascia, skin, amniotic membrane, ligaments, tendons, heart valves, and

blood vessels that do not belong to organs according to the Act on Human Tissue Safety and Management.¹⁾ In human tissue transplantation, the quality of a graft is important to effectively reconstruct defective tissue and facilitate functional recovery. However, considering the risk of disease transmission of malignant tumors or infections after human tissue transplantation, the safety of the use of human tissue is a prerequisite.

Currently, tests for hepatitis B, hepatitis C, AIDS, and syphilis are conducted in the Republic of Korea on tissue donors to determine whether they carry infectious

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disease before collecting bone tissue in compliance with the Act on Human Tissue Safety and Management. Given the incubation period of these viral diseases, a secondary blood test or nucleic acid amplification test (NAT) is also performed 90 days afterwards. A secondary blood test can be done at a relatively low cost, but it is inconvenient for the donor to revisit after a few months. Bone tissue that has not been subjected to a secondary blood test can be discarded if it is not suitable according to the guidelines. The advantages of NAT are its high sensitivity and specificity, and rapid results, but the high cost is a disadvantage.²⁾

Our institution introduced NAT in 2016. We compared and analyzed the effectiveness of the secondary blood test and NAT among different tests for infectious diseases to determine the suitability of allogenic bone grafts.

Table 1. Sex and Retrieval Sites for Each Test

Variable	Secondary blood test	NAT
Sex (M : F)	27 : 155	59 : 211
Retrieval site (hip : knee)	45 : 137	84 : 186

NAT: nucleic acid amplification test.

Table 2. Blood Test Criteria

No.	Test	Results required for confirmation	Remark
Hepatitis B test			
1	HBsAg	Negative	-
2	HBcAb or HBV NAT	Negative	If the HBcAb result is positive, it is determined as nonconfirming or confirmed via additional HBV NAT test.
Hepatitis C test			
3	HCV Ab	Negative	-
4	HCV NAT	Negative	-
AIDS test			
5	HIV 1/2 Ab	Negative	-
6	HIV NAT	Negative	-
Syphilis test			
7	Syphilis (RPR etc.)	Negative	Positive result is confirmed by additional FTA-ABS.

HBsAg: hepatitis B virus surface antigen, HBcAb: hepatitis B core antibody, HBV: hepatitis B virus, NAT: nucleic acid amplification test, HCV Ab: hepatitis C virus antibody, HIV: human immunodeficiency virus, RPR: rapid plasma regain, FTA-ABS: fluorescent treponemal antibody absorbed test.

METHODS

This study was approved by the Institutional Review Board of St. Vincent's Hospital (IRB No. VC20RISI0084). And, informed consent was waived by the Institutional Review Board.

Retrieval of Allogenic Bone

Allogenic bones were retrieved from 452 donors between January 2013 and December 2019. We reviewed the donors' past medical and medication history and excluded cases that did not meet the criteria for allograft suitability such as infectious disease, autoimmune disease, and malignant tumors. Allogenic bones were retrieved from 452 donors including 129 donors undergoing hip replacement arthroplasty and 323 donors treated with knee replacement arthroplasty (Table 1). The average age of the donors was 69 years (range, 33–87 years).

Blood Test and NAT

In total, 182 patients underwent a secondary blood test from January 2013 to December 2015. NAT was performed on 270 patients from January 2016 to December 2019. Tests for hepatitis B virus surface antigen and hepatitis B surface antibody (HBsAg and HBsAb), hepatitis C virus antibody (HCV Ab), AIDS (human immunodeficiency virus [HIV]-1/2-Ab), and syphilis (venereal disease research laboratory or treponema pallidum hemagglutination assay) were performed before bone tissue retrieval to confirm

negative results (Table 2). Given the incubation period of the disease, a secondary blood test was performed at least 90 days after the first blood test. The secondary blood test was performed during a routine examination after hip or knee replacement arthroplasty. NAT is a highly sensitive diagnostic method involving direct amplification of the nucleic acid of the virus and performed within 2 weeks while the patient is hospitalized after the first blood test. NAT has been mandatory by the Ministry of Food and Drug Safety since 2016. The blood sample was collected in a special test tube and sent to Blood Transfusion Research Institute of Korean Red Cross. The results could be notified in 4 to 5 days.

RESULTS

Sixty-seven of the 452 cases (14.8%) were discarded. In the secondary blood test group, 50 out of 182 cases (27.4%), and in the NAT group, 17 out of 270 cases (6.3%) were discarded. Samples in the secondary blood test group were discarded in the event of disagreement on the use of personal information (12%), missed secondary blood tests (6%), confirmation of existing diseases (3.8%), and positive results of microbial tests (1.6%). Samples in the NAT group were discarded in the event of failure to maintain

appropriate temperature during transport (3%), positive results of microbial tests (1.1%), and damaged packaging or nonuse after thawing (1.1%) (Table 3).

In 132 patients who underwent a secondary blood test, none of the donors with a negative first test was positive in the secondary blood test. However, among the 253 cases subjected to NAT, three cases tested positive for HBV although the first blood test was negative.

DISCUSSION

Since the first successful allograft by Dr. William in 1879 for the treatment of osteomyelitis, the use of allograft has increased in a variety of operations for bone tumors and severe fractures and revision arthroplasties.³⁾ Allograft applications have increased in frequency recently, but the risk of contamination and bacterial infection in the process of retrieval, storing, and transplanting of allograft tissue persists. It is very important to reduce the chances of infection during the process, as postoperative infections can lead to fatal outcomes for the recipient.⁴⁾

As a result, the importance of donor screening is becoming more evident. Policies and legislation regulating donor compatibility vary from country to country. It is stipulated that tissue should not be distributed or trans-

Table 3. Reasons for Disposal by Test

Test	Reason for discarding tissue samples	Case
Secondary blood test (50 cases)	Secondary blood test was not performed.	Refusal, 10; Death, 1
		1
	Disagreement with the use of personal information	22
	Confirmation of existing diseases (HBV+, HCV+, R/O HCC, pulmonary tuberculosis)	7
	Positive microbial test results	3
	Dementia diagnosis	2
	Disposal after quality control	2
	Missed prescription	1
	Administration of immunosuppressants	1
	Tissue contamination (drop down)	1
NAT (17 cases)	Positive HBV results in NAT	3
	Failure to maintain appropriate temperatures during transport	8
	Positive microbial test result	3
	Damaged packaging or unused sample after thawing	3

HBV: hepatitis B virus, HCV: hepatitis C virus, HCC: hepatocellular carcinoma, NAT: nucleic acid amplification test.

planted if a donor has infectious diseases, degenerative neurological diseases such as dementia, unclear causes of death, exposure to harmful substances, and potential for cancer cell metastasis. According to the relevant enforcement regulations,⁵⁾ donors with the following conditions are not suitable for tissue retrieval: sepsis, active bacteria or fungal meningitis, tuberculosis, Hansen's disease, malaria, active herpes simplex, diphtheria, scarlet fever, active poliomyelitis, rabies, Reye syndrome, meningitis or encephalitis, malignant tumors, Creutzfeldt-Jakob disease, and Kuru disease. Besides, it is stipulated that tissues from donors with hepatitis B, hepatitis C, AIDS, syphilis, and harmful microorganisms are not suitable for transplantation.

In Korea, a secondary blood test is recommended in addition to the first blood test given the incubation period of infectious diseases according to the existing laws and regulations. However, donors are faced with the hassle of returning to the hospital after a few months for a secondary blood test, and many tissue banks fail to perform secondary blood tests. In some tissue banks, the tissue is discarded, if no secondary blood test is performed. In fact, in our institution, approximately 6% of the donated bone tissue is discarded due to failure in the secondary blood test. The costs incurred in storage, sterilization, and packaging can lead to much higher cost losses; however, it is most important that bone tissue is discarded following a failure in the blood test. Therefore, studies have been conducted to minimize this loss, and some have argued that a secondary blood test is not necessary.⁶⁾ Kappe et al.⁷⁾ reported that no secondary blood test was conducted after 6 months in allograft transplantation, but no infectious disease was found. Harrell et al.⁸⁾ also reported that there were no cases of positive HIV after excluding infected donors based on HIV antibody testing. Hernigou et al.⁹⁾ suggested that radioactive sterilization of allogeneic bone could inactivate HIV.

NAT is an emerging tool to replace the secondary blood tests. NAT can be used to confirm infection by isolating and amplifying nucleic acids (DNA or RNA) directly from a virus in the blood. The cost is higher than that of the current tests, but since the nucleic acid is separated and detected, it reduces the diagnostic window, which is a period in which the antigen and the antibody in the blood cannot be detected due to critically lower levels. Following recent technological advances, various nucleic acid amplification assays have been developed and applied to detect viral infections even before antibody formation. Until late 1999 to early 2000, nucleic acid amplification assays for blood were mandatory in the United States and Europe.

In Korea, enzyme immunity tests and NAT for imported plasma were compulsory according to the Imported Plasma Management Standards established in 2002, and only plasma that is negative for HCV, HIV, and HBV was used for the production of plasma fractionation agents. In June 2012, the revised Raw Plasma Management Standard was expanded and applied not only for imported plasma, but also for domestic raw plasma used in the manufacture of plasma fractions.

Since January 1, 2016, a specific test was used to determine the suitability of transplantation in order to increase the accuracy of blood test results. In particular, NAT was introduced to strengthen the safety management of human tissues. Besides, the Ministry of Food and Drug Affairs has facilitated the cost-effective application of NAT by the Korean Red Cross in order to harmonize related systems and reduce the burden on tissue banks.

Yao et al.¹⁰⁾ reported that morbidity of infectious diseases due to musculoskeletal tissue transplantation is higher than that of infectious diseases caused by blood transfusion, but can be reduced by using NAT. In this study, of the 132 cases subjected to a secondary blood test, none of them was negative in the first blood test but turned positive in the secondary blood test. However, 3 of the 253 cases exposed to NAT were negative in the first test, but positive for HBV.

According to a report published by the Korean Red Cross in 2013 analyzing the effectiveness of HBV NAT, the NAT introduced in 2012 was the most effective among the currently commercially available tests for detecting HBV. The HBV NAT confirmed HBV-positive status in 374 blood samples, which was not evaluated via serological tests from June 12, 2012, to June 11, 2013. This strategy improved the safety of blood management in Korea.

When comparing the effectiveness of a secondary blood test and NAT to determine the suitability of allograft transplantation, it cannot be concluded that the secondary blood test is cost-effective given the discarded bone tissue due to test omission. Besides, the sensitivity and specificity of NAT are higher than that of the secondary blood test. Therefore, it is considered more reasonable to replace the secondary blood test with NAT at a time when the cost of NAT has decreased drastically due to changes in domestic policy.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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