No Detection of Xenotropic Murine Leukemia Virus-Related Viruses in Prostate Cancer in Sanandaj, West of Iran

Mazaher Khodabandehloo1,2*, Weria Hosseini3, Mohammad-Reza Rahmani4, Mohammad-Ali Rezaee4, Mohammad-Saied Hakhamaneshi2,5, Bahram Nikkhoo6, Ali Jalili2,4

Abstract

Background: Multiple etiologies have been hypothesized for prostate cancer, including genetic defects and infectious agents. A recently reported gamaretrovirus, xenotropic murine leukemia virus-related virus (XMRV) has been reported to be detected in prostate cancer. However, this virus has not been detected in similar groups of patients in other studies. Herein, we sought to detect XMRV in prostate cancers and benign controls in Sanandaj, west of Iran. Materials and Methods: In a case-control study, genomic DNA was extracted from formalin fixed and paraffin embedded prostate tissues from a total of 163 Iranian patients. We developed a conventional and a nested PCR assay using primers targeting to an env specific sequence of XMRV. PCR assays were carried out on 63 prostate cancers and 100 benign prostate hyperplasias. Results: Beta-actin sequences were successfully detected in the DNA extracts from all prostate tissues, confirming DNA extraction integrity. We did not detect XMRV in samples either from prostate cancers or benign prostate hyperplasias using XMRV specific primers. Conclusions: We conclude that in our population XMRV does not play a role in genesis of prostate cancer.

Keywords: XMRV - MLV - prostate cancer - Iran

Introduction

Prostate cancer is the most common malignancy in men worldwide (Groom et al., 2012). The annual incidence rates of prostate cancer among Iranian population are dramatically higher than in other countries of Asia (Akbari et al., 2008). Multiple etiologies have been hypothesized for the cause of prostate cancers, including genetic defects, infectious agents and associated inflammation (Silverman et al., 2010). Identification of a potential virus as the cause of prostate cancer is very important because it could facilitate management of this disease such as treatment and prevention. A recently described retrovirus, the xenotropic murine leukemia virus-related virus (XMRV) was detected in about 40% of familial prostate cancer using a DNA microarray (Virochip) containing oligonucleotides comprising conserved sequences from known viral genomes and was strongly associated with homozygosity for the R462Q reduced activity, a variant of the antiviral enzyme, RNase L (Urisman et al., 2006). Murine leukemia viruses (MLVs) belong to gamaretroviruses in the retroviridae family, which viral RNA is reverse-transcribed to DNA during replication. They are endogenous viruses that have integrated their provirus DNA into the mouse genome and can cause leukemias, lymphomas, neurologic diseases, and immunodeficiency disorders in their hosts (Rezaei et al., 2013). Xenotropic MLV (XMLV) replicates only in non-mouse hosts, and ecotropic murine leukemia virus (EMLV) replicates only in mice, but polytropic murine leukemia virus (PMLV) has wider host range. The envelope (env) sequences of XMRV were being so related to endogenous XMLV, hence the naming of the new virus as XMRV. There is about 96% nucleotide sequence homology between XMRV and MLV in the genome (Silverman et al., 2010; Zhou et al., 2012; Rezaei et al., 2013). Also, conformational and cross-reactive epitopes are shared between mouse gamaretroviruses and XMRV. Since original report, some researchers have detected XMRV in human prostate cancers, while others did not (Schlaberg et al., 2009; Arnold et al., 2010; Danielson et al., 2010; Verhaegh et al., 2011). Schlaberg et al. (2009) in USA showed higher prevalence of XMRV DNA by PCR (6%) and viral proteins by immunohistochemistry (IHC) (23%) in tissues from prostate cancers in comparison to noncancerous controls (Schlaberg et al., 2009). A similar study reported, a 22% XMRV prevalence in prostate cancers from Texas but the virus was found in both tumor and non-tumor tissues (Danielson et al., 2010). Moreover neutralizing antibodies for XMRV were identified in 11 of 40 patients with prostate cancer and XMRV sequences were confirmed in five of 11 by nested
PCR and fluorescence in situ hybridization (Arnold et al., 2010; Switzer et al., 2011). Recently a study reported that, XMRV was detectable in some patients with prostate cancer in Kerman, southeast of Iran (Reza et al., 2012). In contrast, in other studies have been found very low or no XMRV prevalence in prostate cancers in Europe (Germany, and the Netherlands) and USA using only RT-PCR (Fischer et al., 2008; Hohn et al., 2009; Aloia et al., 2010; Silverman et al., 2010; Sakuma et al., 2011; Sfanos, 2011; Stieler et al., 2011). Moreover other findings imply that XMRV does not present in humans, and is only seen in the laboratory contamination (Sfanos et al., 2012). Thus, the role of XMRV in human pathogenesis is controversial (Switzer et al., 2011). XMRV was also reported to be presence in patients with chronic fatigue syndrome (CFS) (Lombardi et al., 2009). However, the link between XMRV and CFS is unclear, because in other studies it has failed to detect XMRV infection in CFS patients (Erlewein et al., 2010; Groom et al., 2010; Switzer et al., 2010; van Kuppeveld et al., 2010; Knox et al., 2011; Satterfield et al., 2011; Shin et al., 2011). However, gammaretroviruses are known to induce cancer in animals, understanding XMRV or related MLVs infections in human prostate cancer tissues will shed light on their potential contribution to human disease.

Proposed reasons to explain the conflicting data are unknown but may be: technical differences, lack of standardized XMRV PCR assays, assay sensitivity, contamination by and cross-reactivity of XMRV PCR assays with closely related endogenous MLVs such as trace quantities of mouse genomic DNA found in reagents and samples (Hue et al., 2010; Oakes et al., 2010; Robinson et al., 2010; Sato et al., 2010; Knox, Carrigan et al., 2011; Tuke et al., 2011), differences in the geographical distribution of XMRV, sequence differences among XMRV genomes (Silverman et al., 2010; Singh et al., 2010; Knox et al., 2011) and factors related to the population genetic factors (Switzer et al., 2011).

Due to public health and medical consequences of potential XMRV or related MLVs infection in humans, we considered it is important to confirm or reject their potential XMRV or related MLV infections in human prostate cancer tissues will shed light on their potential contribution to human disease.

Tissue deparaffinization
Prostate tissue sections with 10 µm thicknesses were deparaffinized using Geneth Bio kit (Korea), according to manufacturer’s instruction. Briefly, prostate tissue sections were thawed into 1ml xylene at 50-60°C for 15 minutes. Then supernatant was separated by centrifugation at 13000 rpm and repeated 3-4 times and washed in 100%, 90%, 70%, 50% ethanol for 5 minutes.

DNA extraction
DNAs were extracted using PrimePrep™ genomic DNA isolation kit (GenetBio, Korea) from formalin fixed and deparaffinated prostate tissue sections. Genomic DNA of YAC-1 cells containing MLV provirus, was used as positive control purchased from national cell bank, Iran, Pasteur institute (NCBI Code: C552). Nucleic acid concentrations were determined by spectrophotometry.

Molecular detection of XMRV
Prostate tissue specimens were screened for XMRV and MLV provirus by nested PCR using primers targeting to an XMRV specific envelope (env) sequence, a sequence conserved amongst MLVs. Briefly, we used a first set of primers that encompasses an 1070bp, and a second set of primers to amplify 300bp of env region of the XMRV. The first round (external) primer sequences were as: F1: 5'-AAC AGC ATG GAA AGT CCA GC-3'; R1: 5’-CGT GAT TCC ACT TCT TCT GG-3’. The second round (internal) primer sequences were as: F2: 5’-GGA CGA TGA CAG ACA CTT TC-3’; R2: 5’-GAG TGT TTC CTC GCT TAA GG-3’. In addition, a conventional PCR assay using only second set (internal) primers to amplify 300 bp sequence was done to exclude any detection loss due to possible fragmentation of extracted long DNA molecules from pathologic blocks.

PCR master mix (CinnaGen, Tehran, Iran) was a premixed 2X concentrated solution of 0.04U/µl Taq DNA Polymerase (recombinant), reaction buffer, 3mM MgCl2, 0.4mM of each dNTPs. Primers were added as 10pM F1, 10pM R1 in 1.5µl each in first reaction, and 10pM F2, 10pM R2 1.5µl each in second PCR reaction. 2µl volumes of DNA were used as a template and the final volume of PCR reactions was 20µl. PCR reactions were heated to 94°C for 10 minutes and then cycled 35 times through 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 45 seconds. Final extension 72°C for 10 minutes and was hold in 4°C. Nested PCR products for XMRV and MLV env region would be about 1070 bp and 300 bp DNA bands. Negative controls consisted of reactions with H2O in place of DNA templates. All PCR products were visualized on Ethidium Bromide-stained 1.5% agarose gels. PCR products from the first round and second-round PCR of the correct size were photographed. All specimen preparation, DNA extraction, and PCR testing was done in physically isolated rooms for contamination prevention.

Materials and Methods

Study population and specimen
63 prostate adenocarcinoma cancers and 100 BPH patients were used in this case-control study. Samples were taken from paraffin embedded blocks archived in pathology laboratory, Tohid hospital, Sanandaj, capital of Kurdistan province, in the west of Iran, during 2010-2012. To reduce the exposure of patient samples to potential sources of mouse, XMRV and MLV DNA, standard laboratory procedures for sterile DNA extraction were practiced when handling and processing of specimens.

Beta-actin PCR
To verify the integrity and quality of genomic DNA purified from prostate tissues and to rule out the presence
of PCR inhibitors, β-actin sequence was amplified. β-actin primers were designed as: β-actin F: 5′-GCG TTA CAC CCT TTC TTG AC-3′, β-actin R: 5′-TTG TGA ACT TTG GGG GAT GC-3′; β-actin PCR program was as: initial denaturation at 94°C for 10 min; 94°C for 3 min, 58°C for 30 seconds and 72°C for 45 seconds (35 cycles). Final extension 72°C for 10 minutes and was held at 4°C. It was done for all of the prostate tissue DNA extracts before main XMRV PCRs.

Results

The average age of the prostate patients was 70.77 years, ranged from 33-88 years old. Using the Gleason system, 39.7% of the study population had grade <5 cancer, 25.4% had grade 5-6 cancer, 14.26% had grade 7, and 20.64% had high grade carcinoma. Of total 163 samples, 69 were from rural and 94 from urban; and of cancer cases 28 were from rural and 35 from urban regions.

Beta-actin sequences were successfully detected in the DNA extracts from all prostate tissues, confirming DNA extraction integrity. Designed XMRV and MLV specific primers were able to detect MLV provirus in YAC-1 cell. But testing of DNA extracted from all prostate tissue sections of total 163 specimens including 63 prostate cancers and 100 BPH was negative for XMRV and MLV sequences in nested PCR using external and internal primers targeting to an XMRV and MLV specific envelope (env) sequence, a sequence conserved amongst XMRV and MLVs. Consistently, all of the samples were negative using XMRV and MLV specific internal primers. Thus, the overall prevalence for XMRV and MLV in our samples was 0%. The size of the beta-actin amplimer (180bp) was designed to be similar to the expected amplimer size for PCR detection of XMRV and MLV-related viruses using internal primers. A representative stained gels following doing of PCR assays are shown in Figures 1 and 2.

Discussion

Our finding is consistent with studies demonstrating no association between XMRV and related gammaretroviruses in prostate cancers. Only MLV env sequences were detected in DNA extraction of YAC-1 mouse cell lines. Beta-actin sequences were also detected by PCR in the DNA extractions from all prostate tissues, confirming their DNA integrity and sensitivity of DNA amplification. The reasons of failure to detect XMRV sequences in our specimens are unlikely due to the methods used, such as DNA extraction and PCR amplification, because both beta-actin in all prostate specimens and the env region of MLV in YAC-1 cells were successfully amplified.

Because of the high degree of homology between XMRV and related MLV provirus sequences present in mouse genomic DNA, it has been shown that XMRV specific PCR assays can cross-react with MLV provirus sequences (Urisman et al., 2006; Silverman et al., 2010; Zhou et al., 2012; Rezaei et al., 2013). PCR assays described herein detected MLV provirus env sequences present in mouse genomic DNA (YAC-1 cells) agrees with result of other studies (Danielson et al., 2010; Tang et al., 2012; Rezaei et al., 2013).

A high prevalence of XMRV has been reported in patients with prostate cancer and chronic fatigue syndrome (CFS) in the USA (Urisman et al., 2006; Schlaberg et al., 2009; Arnold et al., 2010). But, in Europe (Fischer et al., 2008; Hohn et al., 2009; Verhaegh et al., 2011), the USA, and recent study in Australia detection of XMRV in prostate (Aloia et al., 2010; Switzer et al., 2010; Rezaei et al., 2013), and breast cancer tissues (Khan et al., 2012) have been failed. Herein, we did not detected XMRV and related MLVs DNA in 163 samples of prostate cancer and BPH, which is in consistence with other reports from the USA, Europe, central Africa, China, and Australia (Simmons et al., 2011; Rezaei et al., 2013; Williams et al., 2013). The reasons for this variation in results might be factors such as, geographic distribution, genetic variations of the virus (Hohn et al., 2009; Luczkowiak et al., 2012) and absence of XMRV and related MLVs in human infections. It is also possible that the quality of the specimens were low due to preservation, handling, and the duration of storage prior to DNA isolation.

Several laboratory methods were applied to detect XMRV in prostate cancers; the results of them do not support any association between XMRV and related gammaretroviruses in prostate cancer (Mendoza et al., 2012; Rezaei et al., 2013). As well, the absence of XMRV or
related MLV may not be due to decreased sensitivity of PCR assay. Since we detected MLV provirus in YAC-1 cells using same primers.

Mouse DNA contamination in laboratory reagents was also reported to be the source of XMRV in studies of prostate cancer tissues and CSF (Switzer et al., 2011; Tang and Hewlett, 2011; Tang et al., 2012) and resulted to retraction of some papers. These results are important science laboratory contamination by endogenous MLVs would be false-positive XMRV detection due to the high homology in DNA sequences between these viruses (Tang and Hewlett, 2011). In addition, primers originally used for XMRV detection were also able to detect homolog MLV sequences from mouse DNA. In a study the absence of XMRV DNA or neutralizing anti-XMRV antibodies suggests no or very low prevalence of XMRV in their cohorts. They conclude that real-time PCR and IHC positive samples were laboratory contamination and false-positive immune reactions (Sakuma et al., 2011). Our PCR result in specimens was negative for XMRV and related MLV, but only YAC-1 cells was positive for MLV; thus we did not have any mouse cross contamination.

Potential limitations of our study are that, due to the limited amount of samples we were unable to test more prostate cancer patients. For viremia examination, unfortunately, it was not achievable to extract RNA from plasma specimens of our patients to analyze by RT-PCR. As well, we were not able to do serologic tests for antibodies that are important features of retroviral infections for additional evidence for non tissue-specific infections. In addition, studies failing to detect XMRV or related MLVs in prostate cancer and CFS patients outnumber the studies confirming the original findings (Korn et al., 2012; Mi et al., 2012; Paolucci et al., 2012; Rezaei, Hearps et al., 2013). In a study the presence of XMRV was detected among some patients with prostate cancer in the Iran, but did not find any correlation between XMRV infection and various clinical pathological parameters of prostate cancer (Reza, Fahimeh et al., 2012).

In conclusions, several studies have used primers that simultaneously detect XMRV and related MLVs in prostate cancers (Hohn et al., 2009; Aloia et al., 2010; Robinson et al., 2011; Switzer et al., 2011; Das Gupta et al., 2012; Rezaei et al., 2013). But, XMRV or related gammaretroviruses (MLVs) were not detected in our samples. We conclude that in our population XMRV or related MLVs does not play a role in tumorigenesis of prostate cancer and adds additional evidence to this area.

Acknowledgements

We would like to thank Kurdistan University of Medical Sciences for financial support and Pathology laboratory of Tohid hospital, Sanandaj, Iran, for preparing prostate specimen and tissue sectioning

References

Akbari ME, Hosseini SJ, Rezaee A, et al (2008). Incidence of genitourinary cancers in the Islamic Republic of Iran: a survey in 2005. Asian Pac J Cancer Prev, 9, 549-52.

Aloia AL, Sfanos KS, Isaacs WB, et al (2010). XMRV: a new virus in prostate cancer? Cancer Res, 70, 10028-33.

Arnold RS, Makarova NV, Osunkoya AO, et al (2010). XMRV infection in patients with prostate cancer: novel serologic assay and correlation with PCR and FISH. Urol, 75, 755-61.

Danielson BP, Ayala GE, Kimata JT (2010). Detection of xenotropic murine leukemia virus-related virus in normal and tumor tissue of patients from the southern United States with prostate cancer is dependent on specific polymerase chain reaction conditions. J Infect Dis, 202, 1470-7.

Das Gupta J, Luk KC, Tang N, et al (2012). Absence of XMRV and Closely Related Viruses in Primary Prostate Cancer Tissues Used to Derive the XMRV-Infected Cell Line 22Rv1. PLoS One, 7, 36072.

Erlwein O, Kaye S, McClure MO, et al (2010). Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome. PLoS One, 5, 8519.

Fischer N, Hellwinkel O, Schulz C, et al (2008). Prevalence of human gammaretrovirus XMRV in sporadic prostate cancer. J Clinical Virology, 43, 277-83.

Groom HC, Boucherit VC, Makinson K, et al (2010). Absence of xenotropic murine leukaemia virus-related virus in UK patients with chronic fatigue syndrome. Retrovirology, 7, 10.

Groom HC, Warren AY, Neal DE, et al (2012). No evidence for infection of UK prostate cancer patients with XMRV, BK virus, trichomonas vaginalis or human papilloma viruses. PLoS One, 7, 34221.

Hoh O, Krause H, Barbarotto P, et al (2009). Lack of evidence for xenotropic murine leukemia virus-related virus (XMRV) in German prostate cancer patients. Retrovirology, 6, 92.

Hue S, Gray ER, Gall A, et al (2010). Disease-associated XMRV sequences are consistent with laboratory contamination. Retrovirology, 7, 111.

Khan G, Philip PS, Naase M, et al (2012). No evidence for the involvement of XMRV or MCV in the pathogenesis of breast cancer. Br J Cancer, 106, 1166-70.

Knox K, Carrigan D, Simmons G, et al (2011). No evidence of murine-like gammaretroviruses in CFS patients previously identified as XMRV-infected. Science, 333, 94-7.

Korn K, Reil H, Ensser A, et al (2012). No evidence of XMRV infection in immunocompromised patients and HIV-positive individuals from Germany. Infection, [Epub ahead of print].

Lombardi VC, Ruscelli FW, Das Gupta J, et al (2009). Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science, 326, 585-9.

Luczkiwiaj K, Martinez-Prats L, Sierra O, et al (2012). Lack of the detection of XMRV or polytropic MLV-related sequences in blood cells from HIV-1-infected patients in Spain. J Acquir Immune Defic Syndr, 59, 101-4.

Mendoza R, Silverman RH, Klein EA, et al (2012). No biological evidence of XMRV in blood or prostatic fluid from prostate cancer patients. PLoS One, 7, 36073.

Mi Z, Lu Y, Zhang S, et al (2012). Absence of xenotropic murine leukemia virus-related virus in blood donors in China. Transfusion, 52, 326-31.

Oakes B, Tai AK, Cingoz O, et al (2010). Contamination of human DNA samples with mouse DNA can lead to false detection of XMRV-like sequences. Retrovirology, 7, 109.

Paolucci S, Piralla A, Zanello C, et al (2012). Xenotropic and polytropic murine leukemia virus-related sequences are not detected in the majority of patients with chronic fatigue syndrome. New Microbiol, 35, 341-4.

Reza MA, Fahimeh G, Reza MH (2012). Evaluation of xenotropic murine leukemia virus and its R426Q polymorphism in patients with prostate cancer in Kerman, southeast of Iran. Asian Pac J Cancer Prev, 13, 3609-73.

Rezaei SD, Hearps AC, Mills J, et al (2013). No association...
between XMRV or related gammaretroviruses in Australian prostate cancer patients. Virol J, 10, 20.

Robinson MJ, Erlwein OW, Kaye S, et al (2010). Mouse DNA contamination in human tissue tested for XMRV. Retrovirology, 7, 108.

Robinson MJ, William Tuke P, Erlwein O, et al (2011). No Evidence of XMRV or MuLV Sequences in Prostate Cancer, Diffuse Large B-Cell Lymphoma, or the UK Blood Donor Population. Advances in Virology, 2011, 1-6.

Sakuma T, Hue S, Squillace KA, et al (2011). No evidence of XMRV in prostate cancer cohorts in the Midwestern United States. Retrovirology, 8, 23.

Sato E, Furuta RA, Miyazawa T (2010). An endogenous murine leukemia viral genome contaminant in a commercial RT-PCR kit is amplified using standard primers for XMRV. Retrovirology, 7, 110.

Satterfield BC, Garcia RA, Jia H, et al (2011). Serologic and PCR testing of persons with chronic fatigue syndrome in the United States shows no association with xenotropic or polytropic murine leukemia virus-related viruses. Retrovirology, 8, 12.

Schlaberg R, Choe DJ, Brown KR, et al (2009). XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. Proc Natl Acad Sci USA, 106, 16351-6.

Sfianos KS, Aloia AL, De Marzo AM, et al (2012). XMRV and prostate cancer—a “final” perspective. Nat Rev Urol, 9, 111-8.

Sfianos KS, Isaacs JT (2011). The “infectious” nature of human prostate cancer: a cautionary note. Oncotarget, 2, 281-3.

Shin CH, Bateman L, Schlaberg R, et al (2011). Absence of XMRV and other MLV-related viruses in patients with Chronic Fatigue Syndrome. J Virol, 85, 7195-202.

Silverman RH, Nguyen C, Weight CJ, et al (2010). The human retrovirus XMRV in prostate cancer and chronic fatigue syndrome. Nat Rev Urol, 7, 392-402.

Simmons G, Glynn SA, Holmberg JA, et al (2011). The Blood Xenotropic Murine Leukemia Virus-Related Virus Scientific Research Working Group: mission, progress, and plans. Transfusion, 51, 643-53.

Singh IR, Gorzynski JE, Drobysheva D, et al (2010). Raltegravir is a potent inhibitor of XMRV, a virus implicated in prostate cancer and chronic fatigue syndrome. PLoS One, 5, 9948.

Stieler K, Schindler S, Schlomm T, et al (2011). No detection of XMRV in blood samples and tissue sections from prostate cancer patients in Northern Europe. PLoS One, 6, 25592.

Switzer WM, Jia H, Hohn O, et al (2010). Absence of evidence of xenotropic murine leukemia virus-related virus infection in persons with chronic fatigue syndrome and healthy controls in the United States. Retrovirology, 7, 57.

Switzer WM, Jia H, Zheng H, et al (2011). No association of xenotropic murine leukemia virus-related viruses with prostate cancer. PLoS One, 6, 19065.

Tang N, Frank A, Leckie G, et al (2012). Development of sensitive single-round pol or env RT-PCR assays to screen for XMRV in multiple sample types. J Virol Methods, 179, 127-34.

Tang S, Hewlett IK (2011). Testing strategies for detection of xenotropic murine leukemia virus-related virus infection. Adv Virol, 2011, 281425.

Tuke PW, Tettmar KI, Tamuri A, et al (2011). PCR master mixes harbour murine DNA sequences. Caveat emptor! PLoS One, 6, 19953.

Urisman A, Molinaro RJ, Fischer N, et al (2006). Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog, 2, 25.

van Kuppevelt FJ, de Jong AS, Lanke KH, et al (2010).

DOI:http://dx.doi.org/10.7314/APJCP.2013.14.11.6929

No Detection of Xenotropic MRV in Prostate Cancer in Iran

Prevalence of xenotropic murine leukaemia virus-related virus in patients with chronic fatigue syndrome in the Netherlands: retrospective analysis of samples from an established cohort. BMJ, 340, 1018.

Verhaegh GW, de Jong AS, Smit FP, et al (2011). Prevalence of human xenotropic murine leukemia virus-related gammaretrovirus (XMRV) in dutch prostate cancer patients. Prostate, 71, 415-20.

Williams DK, Galvin TA, Gao Y, et al (2013). No evidence of xenotropic murine leukemia virus-related virus transmission by blood transfusion from infected rhesus macaques. J Virol, 87, 2278-86.

Zhou Y, Steffen I, Montalvo L, et al (2012). Development and application of a high-throughput microneutralization assay: lack of xenotropic murine leukemia virus-related virus and/or murine leukemia virus detection in blood donors. Transfusion, 52, 332-42.