Situation of XMRV and Blood Transfusion

Celso Bianco, MD
ISBT Working Party on TTID
Lisbon, June 19, 2011
Detection of an Infectious Retrovirus, XMRV, in Blood Cells of Patients with Chronic Fatigue Syndrome

Vincent C. Lombardi,1* Francis W. Ruscetti,2* Jaydip Das Gupta,3 Max A. Pfost,1 Kathryn S. Hagen,1 Daniel L. Peterson,1 Sandra K. Ruscetti,4 Rachel K. Bagni,5 Cari Petrow-Sadowski,6 Bert Gold,2 Michael Dean,2 Robert H. Silverman,3 Judy A. Mikovits1‡
Conclusions: CFS and XMRV

- XMRV found in 67% of CFS patients
- An immune response to the virus was detected in some CFS patients
- Data suggest that the human population is at risk from infection with XMRV (3.7% of controls are DNA positive)
- *Given that infectious virus is present in plasma and in blood cells, blood-borne transmission is a possibility.*

**Common Names**

- Chronic Fatigue Syndrome (CFS, U.S. Health and Human Services Committee)
- Myalgic encephalomyelitis (ME, EU)
- Chronic fatigue and immune dysfunction syndrome (CFIDS, US Association)
- Post-viral fatigue syndrome
- Post-infectious fatigue syndrome
- X-associated neuroimmune disease (XAND)
- Worldwide prevalence 0.4-1%; US 1.2-4 million individual
• CFIDS
  – http://www.cfids.org/default.asp

• CFIDS Research 1st
  – http://www.research1st.com/2011/06/01/xmrv-trials-and-tribulations/

• Position on blood donation
  – There are numerous medical reasons why people with CFS should not donate blood…. The CFIDS Association of America has long advised against CFS patients donating blood [or organs].”
Xenotropic murine leukemia virus–related virus (XMRV) and blood transfusion: report of the AABB interorganizational XMRV task force


TRANSFUSION 2011;51:654-661
“…AABB recommends that blood collecting organizations make educational information available regarding the reasons why an individual diagnosed with CFS should not donate blood.…”
Resources from AABB

• AABB Bulletin, June 18, 2010 recommends educational materials discouraging individuals with CFS from donating blood

• Fact Sheet (March 2011)

• Table of Published Studies (April 27, 2011)
• Analytical NAT panel development:
  – analysis of labs with assays (CDC, FDA, NCI, WPI, BSRI) abilities to find/quantify XMRV from productive cell line in blinded positive and control panel in spiked whole blood and plasma samples

• Clinical NAT panel development:
  – Plasma and whole blood panels for XMRV prevalence in 400 blood donors from BSRI (Reno), 25 XMRV pos. CFS patients from WPI, 25 controls (+ and -) each

• Serology validation after nucleic acid

• Epidemiology study design
Phase I Analytical Panel - Results

### Whole Blood Panel

<table>
<thead>
<tr>
<th>Proviral copies/ml</th>
<th>CDC</th>
<th>FDA(H)</th>
<th>FDA(L)</th>
<th>GP</th>
<th>NCI</th>
<th>WPI</th>
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### Plasma Panel

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<th>Viral RNA copies/ml</th>
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<th>FDA(L)</th>
<th>GP</th>
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<th>WPI</th>
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</table>

Legend:
- 0/3 (for dilution series) or 0/6 (for negatives) replicates called positive
- 1/12 negative controls called positive (panels run twice - once each by two different operators)
- 1/6 negative controls called positive (identified as non-specific band of human genomic origin by sequencing subsequent to decoding of results)
- 1/3 replicates called positive
- 2/3 replicates called positive
- 3/3 replicates called positive
## Summary of Phase IIb NAT and Antibody Results

<table>
<thead>
<tr>
<th>Subject ID (description)</th>
<th>NCI</th>
<th>G-P</th>
<th>CDC</th>
<th>WPI</th>
<th>NCI* Ab</th>
<th>CDC Ab</th>
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<td>Subject 1 - Plasma - day 0</td>
<td>Negative</td>
<td>Non reactive</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
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<td>Non reactive</td>
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<td>Subject 1 - PBMC - day 0</td>
<td>Negative</td>
<td>Non reactive</td>
<td>Negative</td>
<td>Positive</td>
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<tr>
<td>Subject 1 - PBMC - day 2</td>
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<td>Non reactive</td>
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<td>Subject 2 - Plasma - day 0</td>
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<td>Negative</td>
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<td>Subject 2 - Plasma - day 2</td>
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<td>Non reactive</td>
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<td>Subject 3 - Plasma - day 0</td>
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<td>Non reactive</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
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<td>Subject 3 - Plasma - day 2</td>
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<td>Negative</td>
<td>Negative</td>
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<td>Subject 3 - PBMC - day 0</td>
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<tr>
<td>Subject 3 - PBMC - day 2</td>
<td>Negative</td>
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<td>Positive</td>
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<td>Subject 4 - Plasma - day 0</td>
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<td>Subject 4 - Plasma - day 2</td>
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<td>Subject 4 - PBMC - day 0</td>
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<td>Negative</td>
<td>Non reactive</td>
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<td>Pedigreed Negative - Plasma - day 0</td>
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<td>Non reactive</td>
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<td>Pedigreed Negative - Plasma - day 2</td>
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<td>Non reactive</td>
<td>Negative</td>
<td>Negative</td>
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<td>Negative</td>
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<tr>
<td>Pedigreed Negative - PBMC - day 0</td>
<td>Negative</td>
<td>Non reactive</td>
<td>Negative</td>
<td>Positive</td>
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<tr>
<td>Pedigreed Negative - PBMC - day 2</td>
<td>Negative</td>
<td>Non reactive</td>
<td>Negative</td>
<td>Negative</td>
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</tbody>
</table>

* Ruscetti
Blood XMRV Scientific Research Working Group Activities

- Lombardi et al published
- SRWG established
- First SRWG meeting
- Virus samples for quantitation distributed
- Negative samples distributed
- Phase I panel distributed
- Phase Ia panel collected
- Phase Ia panel distributed
- Phase IIa panel collected
- Phase IIa panel distributed
- Presentation of findings

- CDC reports
- NCI/DPR reports
- FDA/WPI reports
- FDA (Hewlett) reports
- NCI/DPR reports
- CDC reports
- WPI reports
- NCI/DPR reports
- CDC reports
- GenProbe reports
- NCI/DPR reports
- CDC reports
- WPI partially reports
- NCI Russett reports

BPAC, XMRV Conf., AABB, BPAC
COMMENTSARY


TRANSFUSION 2011;51:643-653.
Question for the Blood Products Advisory Committee of FDA (December 14, 2010):

Do the scientific data support asking donors about a medical history &/or diagnosis of CFS as a basis for indefinite deferral?

9 yes, 4 no, no abstentions
Disease-associated XMRV sequences are consistent with laboratory contamination

Stéphanie Hui1, Eleanor R Gray1, Astrid Gaill1, Anti Katsoulakis1, Chun Ping Tan1, Charlotte J Haucke2, Stuart McLean1, Deenan Pillay1, Andrew Futreal2, Jeremy A Grant1, Oliver G Pybus3, Paul Kellam4, Greg J Havens5

Abstract

Background: Xeroropic myeloid leukemia virus (XMRV) is a gammaretrovirus that has been associated with prostate cancer. However, the evidence for its association with prostate cancer is not strong. The aim of this study was to determine whether contamination of patient samples by XMRV can lead to false detection of XMRV-like sequences.

Results: We used a PCR-based approach to study the prevalence of XMRV-like sequences in patient samples. We found that XMRV-like sequences were only detected in patient samples that had been contaminated with XMRV. The detection of XMRV-like sequences was significantly higher in patient samples that had been contaminated with XMRV than in patient samples that had not been contaminated with XMRV (p = 0.006). In addition, we found that XMRV-like sequences were only detected in patient samples that had been contaminated with XMRV and that the detection of XMRV-like sequences was significantly higher in patient samples that had been contaminated with XMRV than in patient samples that had not been contaminated with XMRV (p = 0.006).

Conclusions: Our results suggest that contamination of patient samples by XMRV can lead to false detection of XMRV-like sequences. Therefore, it is important to use appropriate controls to detect XMRV-like sequences in patient samples.

An Endogenous Murine Leukemia Viral Genome Contaminant in a Commercial RT-PCR Kit is Amplified Using Standard Primers for XMRV

Eiji Sato1, Rika A Funada1, Takao M. Miyazawa1

Abstract

During pilot studies to investigate the presence of viral RNA of xenotropic murine leukemia virus (XMRV) in human cells, we observed a positive signal in the RT-PCR assay. We investigated the cause of this positive signal and found that it was due to the presence of a contaminant genome of XMRV in the RT-PCR kit.

Results: We investigated the cause of the positive signal in the RT-PCR assay and found that it was due to the presence of a contaminant genome of XMRV in the RT-PCR kit. We also found that the contaminant genome of XMRV was amplified using standard primers for XMRV.

Conclusions: The presence of a contaminant genome of XMRV in the RT-PCR kit can lead to false detection of XMRV-like sequences. Therefore, it is important to use appropriate controls to detect XMRV-like sequences in patient samples.

Contamination of human DNA samples with mouse DNA can lead to false detection of XMRV-like sequences

Brendan Oakes1, Albert K Tai1, Oya Cingoz1, Madeleine H Herfield1, Susan Levine1, John M Coffin4, Brigitte T Huber5

Abstract

Background: In 2006, a novel gammaretrovirus, XMRV (xenotropic murine leukemia virus-related virus), was discovered in some prostate tumors. A recent study indicated that this infectious retrovirus can be detected in 87% of patients suffering from chronic fatigue syndrome (CFS), but only very few healthy controls (4%). However, several groups have published in 2006 that they could not identify XMRV DNA or RNA sequences in other cohorts of CFS patients, while another group detected murine leukemia virus (MLV)-like sequences in 87% of such patients but only 7% of healthy controls. Since there is a high degree of similarity between XMRV and abundant endogenous MLV proviruses, it is important to distinguish contaminating mouse sequences from true infections.

Results: DNA from the peripheral blood of 112 CFS patients and 16 healthy controls was tested for XMRV with two different PCR assays. A Tadbash PCR assay specific for XMRV pol sequences was highly specific and detected viral DNA from 2 XMRV-infected cells (< 10-10 pg DNA) in up to 5 µg of human genomic DNA, but yielded negative results in the test of 600 ng genomic DNA from 10000 peripheral blood cells of all samples tested. However, positive results were obtained with one of these samples, using a less specific nested PCR assay for a different XMRV sequence. This PCR assay revealed a wide variety of virus-related sequences, some identical to those found in prostate cancer and CFS patients, others more closely related to known endogenous MLVs. However, all samples tested negative for XMRV and/or MLV DNA were also positive for human HLA-A allele-specific A-type particles (ApA) long terminal repeat and most were positive for murine microsatellite cytomegalovirus sequence. No contamination was observed in any of the negative control samples, containing those with no DNA template and those that were amplification in each assay.

Conclusions: Mouse cells contain upwards of 100 copies each of endogenous MLV DNA. Even much less than one cell's worth of DNA can yield a detectable product using highly sensitive PCR technology. It is, therefore, vital that contamination from mouse DNA be monitored with adequately sensitive assays in all samples tested.
- qPCR and PCR on buffy coat and whole blood
- ELISA and Western blot on plasma
- Virus culture
- 100 CFS and 200 matched controls
- 14 CFS samples from Reno study
No Evidence of Murine-Like Gammaretroviruses in CFS Patients Previously Identified as XMRV-Infected

Recombinant Origin of the Retrovirus XMRV
On the origin of XMRV

1992 Patient CWR22 CaP (No DNA/RNA)

CWR22 xenograft in nude mice

1992 CWR22 3\textsuperscript{rd} pass. (gDNA)
CWR22 7\textsuperscript{th} pass. (gDNA)
CWR22 unk. pass. (No DNA/RNA)

1996 CWR22 (total nucleic acid)
2152, 2524, 2272, 2274

1999 Cell line 22Rv1 (gDNA)

2001 Cell line CWR R1 (gDNA)

preXMRV 1 + 2 recombination
preXMRV 1 + 2
Late xenografts + cell lines XMRV positive

Pathak et al. CROI 2011.
In the issue of 23 October 2009, Science published the Report “Detection of an infectious retrovirus, XMRV, in blood cell of patients with chronic fatigue syndrome,” a study by Lombardi et al. purporting to show that a retrovirus called XMRV (xenotropic murine leukemia virus-related virus) was present in the blood of 67% of patients with chronic fatigue syndrome (CFS) compared with 3.7% of healthy controls (1). Since then, at least 10 studies conducted by other investigators and published elsewhere have reported a failure to detect XMRV in independent populations of CFS patients. In this week’s edition of Science Express, we are publishing two Reports that strongly support the growing view that the association between XMRV and CFS described by Lombardi et al. likely reflects contamination of laboratories and research reagents with the virus.

The authors of the Lombardi study believe that it is premature to conclude that the negative studies are accurate or change the conclusions of the original studies and we fully agree,” said Annette Whittemore, President of the Whittemore Peterson Institute. (WPI Press release, May 31, 2011)
Ongoing Studies

- NHLBI will continue to support the **XMRV blood study**. Results are expected in the Fall of 2011.
- The Lipkin laboratory-based study is designed to rigorously evaluate whether the presence of XMRV/MLV nucleic acids in the blood is associated with CFS. Researchers, working with clinicians in six regions across the United States, will compare blood and plasma samples from patients diagnosed with CFS to samples from healthy people who have not been diagnosed with CFS and who are matched to the CFS patients by age, sex, and geography. Study results are anticipated later this year.
Proposed REDS-III XMRV Study
Supported by NHLBI

• Blood donor prevalence over four decades using total of 10,000 samples from 4 NIH repositories:
  – Transfusion Transmitted Viruses Study (TTVS)
  – Transfusion Safety Study (TSS)
  – Retrovirus Epidemiology in Donors Study (REDS) General Leukocyte and Plasma Repository (GLPR)
  – Viral Activation by Transfusions Study (VATS)
  – REDS Allogeneic Donor and Recipient Repository (RADAR)

• Transfusion-Transmission using TTVS, VATS and RADAR repositories
  – Rates of transfusion-transmission and correlations of transmission with viral and serologic findings in XMRV+ donations
  – Effect of routine blood filtration (leukoreduction) and blood component storage period on transmission
  – Limited data on mortality and morbidity

• Utilize Abbott and Gen-Probe high-throughput screening assays for serology and NAT
Thank you!
cbianco@americasblood.org