Xenotropic Murine Leukemia Virus-Related Virus as a Case Study: Using a Precautionary Risk Management Approach for Emerging Blood-Borne Pathogens in Canada

Michael G. Tyshenko et al.*
*McLaughlin Centre for Population Health Risk Assessment,
Institute of Population Health, University of Ottawa, Ontario,
Canada*

1. Introduction

In October 2009 it was reported that 68 of 101 patients with chronic fatigue syndrome (CFS) in the United States, when tested, were infected with a novel gamma retrovirus, xenotropic murine leukemia virus-related virus (XMRV) (Lombardi et al., 2009). XMRV is a recently discovered human gammaretrovirus first described in prostate cancers that shares significant homology with murine leukemia virus (MLV) (Ursiman et al., 2006). It is known that XMRV can cause leukemias and sarcomas in several rodent, feline, and primate species but has not been shown to cause disease in humans. XMRV was detectable in the peripheral blood mononuclear cells (PBMCs) and plasma of individuals diagnosed with CFS (Lombardi et al., 2009). After this report was published there was a great deal of uncertainty surrounding this emergent virus and its involvement in the etiology of CFS. The uncertainty was, in part, due to CFS being a complex, poorly understood multi-system disorder with different disease criteria used for its diagnosis. CFS, also known as Myalgic Encephalomyelitis (ME), is a debilitating disease of unknown origin that is estimated to affect 17 million people worldwide. The initial report connecting XMRV to prostate cancers and CFS garnered significant media and scientific interest since it provided a potential

* Susie ElSaadany[2**], Tamer Oraby[1], Marian Laderoute[2], Jun Wu[2], Willy Aspinall[3],
Daniel Krewski[1, 4] and Peter R. Ganz[5]
[1]McLaughlin Centre for Population Health Risk Assessment, Institute of Population Health, University of Ottawa, Ontario, Canada
[2]Blood Safety Surveillance and Health Care Acquired Infections Division, Centre for Communicable Diseases and Infection Control, Public Health Agency of Canada, Ottawa, Ontario, Canada
[3]Aspinall and Associates, Cleveland House, High Street, and Earth Sciences, Bristol University, Bristol, United Kingdom
[4]Department of Epidemiology and Community Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada
[5]Health Canada, Director's Office, Ottawa, Ontario, Canada
** Corresponding Author

explanation for the disease but also an avenue for possible therapeutic treatments since XMRV is known to be susceptible to some anti-retroviral drugs (Cohen, 2011).

The results first reported by Lombardi et al. (2009) suggested that there may be a strong relationship between XMRV infections and CFS. However, other studies later completed from North America (Switzer et al., 2010), Europe (Groom et al., 2010; van Kuppeveld et al., 2010) and China (Hong et al., 2010) failed to find any XMRV associated with CFS patient samples.

Since XMRV was putatively associated with CFS (Lombardi et. al, 2009) and prostate cancer patient groups (Urisman et al., 2006; Schlaberg et al., 2009), these patient groups' blood policies as donor groups were of greatest concern. Individuals previously diagnosed with cancer are already deferred in Canada[1], so the main issue of the need for new policy focused squarely on XMRV and its linkage with CFS. Data from the 2002-2003 Canadian Community Health Survey revealed that about 5% of those surveyed self-reported as having been diagnosed with at least one of the following conditions: CFS, fibromyalgia or multiple chemical sensitivity. Extrapolating from this study it is estimated that approximately 341,000 Canadians self-reported as being diagnosed with CFS. If a confirmed linkage between XMRV and CFS was established this would have a significant impact on policies for blood safety, as well as cells, tissues and organ (CTO) transplantation.

Other retroviruses such as human immunodeficiency virus (HIV) and human T-lymphotropic virus (HTLV) have been shown to infect human cell lines and lymphocytes when the virus is taken from human samples; also these retroviruses are known to be transmitted by transfusion (Lombardi et al., 2009). MLV-like virus was reported in symptomatic patients persisting for over a decade and the detection of XMRV in healthy controls suggested that asymptomatic carriers also likely existed within the donor population (Lo et al., 2010; Lombardi et al., 2009). These research studies highlighted that XMRV could be a potential emerging threat to transfusion/transplantation of blood, cells, tissues and organ safety globally.

Uncertainty over XMRV as an emerging blood-borne pathogen was confounded by considerable information gaps in the available peer review literature. The lack of published research studies in this area was confirmed by a PubMED[2] database search conducted June 1, 2010 using the keywords "XMRV" which resulted in a scant number of 43 peer reviewed papers beginning from the year 2006 when the first paper on this topic was published. The search results highlight how recent this topic area was for researchers and decision-makers alike. Moreover, it reflected how little research and scientific information was available to help inform evidence-based decision making.

By late 2011 the situation surrounding XMRV and its uncertainty as a new emerging pathogen appeared to be largely resolved. Several published scientific studies indicated that XMRV positives reported in previously published cohort studies were likely the result of contamination (Hue et al., 2010; Kaiser, 2011; Carlowe 2011; Smith, 2010). In October 2011

---

[1] Permanently if they are blood donors, and for five years if they are cell tissue or organ (CTO) donors; they can be CTO donors only if there is no evidence of disease return.

[2] NCBI PubMED: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed. PubMED includes over 15 million peer review citations of scientific research articles from the 1950s to present day.

the co-authors of the original research paper published in 2009 that described detection of XMRV in blood cells of patients with CFS (as reported in Lombardi et al., 2009) issued a partial retraction. A re-examination of the samples used showed that some of the CFS PBMC DNA preparations were contaminated with XMRV plasmid DNA (Silverman et al., 2011). The idea of mouse DNA contamination in some laboratories and reagents provides the most parsimonious explanation for the geographic differences found in patient cohort studies completed in different countries and the inability to reproduce XMRV positives.

Sample contamination appeared to stem from multiple sources. In one study, Robinson et al. (2010) analyzed mouse DNA contamination in human tissue samples and also tested for XMRV. The results showed that contamination with mouse DNA was widespread in formalin fixed paraffin embedded human prostate tissue samples detectable by polymerase chain reaction (PCR) assays that targeted a high copy number mouse DNA sequence (intracisternal A particle long terminal repeat DNA). It was also reported by several research laboratories that an endogenous murine leukemia viral genome contaminant was found in a commercial reverse transcriptase-polymerase chain reaction (RT-PCR) kit used in some studies to detect XMRV. In some cases, the PCR "master mixes" used contained small amounts of contaminating mouse DNA sequences that were amplified during testing of human samples. Amplification occurred due to the high degree of sequence similarity between the XRMV oligonucleotide primers used and the contaminating mouse nucleic acids (Bacich et al., 2011; Sato et al., 2010; Tuke et al., 2010; Oakes et al., 2010; Knox et al., 2011).

A re-evaluation of blood samples from 61 patients with CFS by PCR and RT-PCR methods for the detection of viral nucleic acids and assays used to detect infectious virus and virus-specific antibodies revealed no evidence of XMRV. Initially, 43 of these samples had tested positive for XMRV. The results were consistent with previous reports that detected MLV nucleic acid sequences in commercial reagents and that the previous evidence linking XMRV and MLVs to CFS was likely attributable to contamination (Knox et al., 2011).

Other follow-on studies confirmed that XMRV appeared to be a contaminant of prostate cells lines and human tissue samples. Investigation into an existing permanent prostate cell line, 22Rv1 which is widely used as it is one of very few cell lines available to study androgen-independent prostate cancer, revealed that prior to 1996, the cell line did not contain XMRV DNA. The authors suggested the presence of XMRV after this time was likely an introduced artifact (Cohen, 2011; Yang et al., 2011). Moreover, further investigation revealed that XMRV was not present in the original CWR22 tumor used to generate the prostate cell line but it arose by recombination of two overlapping and highly homologous proviruses (PreXMRV-1 and PreXMRV-2) that were present in the mice during tumor passaging. Paprotka et al. (2011) proposed that the association of XMRV with human disease was due to contamination of human samples with virus originating from this recombination event. However, it did not explain earlier outbreaks of CFS-ME which occurred prior to 1996 nor that some of the patients who tested positive for XMRV had disease onset prior to 1996.

Thus, the early differences reported in peer review literature during the 2009 to 2010 time period and the eventual resolution surrounding XMRV as a contamination artifact by mid-2011 provide an interesting case study to review for the application of precautionary action

when managing emerging blood-borne pathogens. The question of "when to act and exercise precaution" given an emerging threat to blood safety with high uncertainty is important for ensuring public health safety for blood transfusions and transplantation of CTOs.

2. Expert discussion for XMRV as an emerging blood-borne pathogen

The 2009-2010 time period presented a significant challenge to health policy requiring management decisions for XMRV as a potential emerging threat to blood and CTO safety. The context was of a lack of available risk information, a lack of an approved diagnostic test for large-scale blood screening, a virus with a relatively high prevalence rate in the general population and defined patient groups (eg., CFS cohorts and prostate cancer groups, as reported by a limited number of research studies), and contradictory research results from different patient clinical samples estimation of XMRV prevalence (e.g., North American versus European XMRV clinical studies). Early risk management decisions must be based on data which is scarce, for a risk that is emerging, highly uncertain and largely unquantified.

In Canada, one of the early risk management steps was to convene a group of experts to discuss the risks associated with XMRV. To address this uncertainty and to inform decision making as a tool to guide public health policy, a half-day workshop was held to discuss the issues and relevant questions about XMRV knowledge gaps. The "International Risk Assessment Workshop Results for XMRV with respect to Blood, Cells, Tissues and Organ Safety Structured Expert Elicitation" policy workshop was held on September 29, 2010 in Ottawa, Canada. The focus was to discuss various XMRV issues within a policy and risk management context.

Experts discussed various related issues including: scientific uncertainty and contradictory XMRV data reported in peer review literature; the prevalence of XMRV; risk parameters affecting XMRV transmission; latency of XMRV; routes of XMRV transmission; risk mitigation of XMRV (the use of leukoreduction); and XMRV disease relationships (whether or not the virus causal or non-causal for CFS, prostate cancer or other diseases).

Expert opinion can be used to address such questions and provide insight into the uncertainties surrounding emerging risks where scientific data is missing, sparse or uninformative. Expert opinion can, at the very least, provide useful indications of possible risks and a robust discussion of pertinent issues for which current data preclude a direct evidence-based assessment (Aspinall, 2010). Such indications can, in turn, inform risk management decision making about emerging risks which must be made even in the face of significant uncertainty. In the absence of more definitive data, expert elicitation can be used to inform policy responses to emerging threats as they occur, and help prioritize issues until scientific research is in a position to support evidenced-based risk management policy development.

3. Scientific uncertainty and contradictory XMRV data

During the expert workshop a number of plausible reasons were postulated to explain the variability in XMRV prevalence data reported in different countries.[3] First, there may be

---

[3] Note that during the time of the workshop (September, 2010) there were no published research studies confirming mouse DNA contamination of patient samples or contamination of diagnostic test kits with minute quantities of mouse genetic material that could result in XMRV false positives.

greater sequence diversity in XMRV variants than originally observed. As a result, PCR oligonucleotide primers with mismatches used in different studies may not amplify XMRV sequence variants as effectively, resulting in missed positive samples.

Second, *in vivo* reservoir(s) of viral replication may not identified suggesting that other tissues may be better for testing than PBMCs. Cells, other than leukocytes, may be the primary target cell infected *in vivo*. Until 2010, most negative XMRV studies used DNA prepared from PBMCs for PCR testing but XMRV virus may be present predominantly as plasma viremia which would be difficult to detect if only these cells were analysed by PCR.

Third, XMRV sequence within the population may be more heterologous than the known published sequences. All negative studies tested for the virus using only the VP62 prototypical prostate XMRV strain sequences. Lombardi et al. (2009) in their study showed XMRV in a high percentage of CFS patients using culture and serology in addition to PCR testing. At the time the experts cautioned that until more was known about viral reservoirs for XMRV, the replication rates and the viral sequence diversity that the use of a single stand-alone assay (such as real time and single round PCR on fresh patient materials) may not be enough to detect true positive cases.

Fourth, the worldwide distribution of XMRV is low and scattered like HTLV-1 making its detection in clinical patient groups difficult to confirm. The low viral load in conjunction with the test procedures used, including the sample's preparation, treatment and storage varied between the different studies which could have affected XMRV detection.

Fifth, patient selection and methods applied to different studies varied widely; this could result in samples from patients who are not true CFS cases. Differences in the patient groups studied that were from different geographic areas and the variability in patients within these groups for CFS disease severity and duration may have affected results. There were and are variations in the different CFS case definitions in use. For example, the most widely used CFS case definition is the Fukuda, USA CDC criteria (Fukuda et al., 1994). Other CFS case definitions used to select research subjects include the Canadian Consensus Criteria for CFS-ME published in 2003 (Carruthers et al., 2003), Holmes et al.'s CFS working case definition (Holmes et al., 1988), the Oxford criteria (Sharpe et al., 1991) and the CDC clinically empirical approach (Reeves et al., 2005).

In hindsight, the variability in XMRV detection was likely due to mouse DNA or VP62 plasmid contamination within samples and/or the use of mouse DNA contaminated RT-PCR kits as later detailed by various studies (Sato et al., 2010; Robinson et al., 2010).

4. Prevalence of XMRV

The experts indicated that estimating an overall worldwide prevalence for XMRV was difficult as differences in regional demographics exist. However, the experts considered the prevalence in the UK, France, USA and Canada to be nearly the same. The experts indicated that there was a clear need for more evidence regarding XMRV incidence, for example completing an age stratified prevalence study or a simple cross-section of prevalence was suggested as a key experiment that would help to inform this answer.

In Canada, the National Microbiology Laboratory (Public Health Agency of Canada, Winnipeg) had undertaken a number of laboratory studies on XMRV, focusing on screening for X/P-MLV related virus sequences in healthy controls and patients with multiple blood transfusions. Groups tested included a small blood bank repository (n = 84), Canadian Blood and Marrow Transplant Group (CBMTG) (n = 54), Canadian Blood Services Registry (n = 76 plasma samples and n = 29 buffy coat samples from hemophiliacs), Canadian Aphaeresis Group - Thrombotic thrombocytopenic purpura patients (n = 23), liver transplant patients (n = 21) and HEV/HCV genotyping samples (n = 94, comprised of 75 HCV and 9 HEV samples). Despite testing a number of at-risk patients and multiply transfused patients with single round PCR methods only 1 patient in the CBMTG group (1.8%) and 3 out of 75 HCV patients (4%) were XMRV positive. All of the other samples showed no evidence of XMRV by nucleic acid testing (Dr. Anton Andonov, Public Health Agency of Canada, personal communication). Results from testing Canadian patients showed little XMRV associated with CFS patient or high risk patient groups.

5. Risk parameters for XMRV

The experts displayed good group agreement on the median age of infection for XMRV of approximately 26 years with a range from 2 to 63 years. The experts believed that the proportion of men to women was 1:1 regarding XMRV infectivity; in other words, the experts did not think that there was a gender bias for XMRV infection within human populations. Position R462Q in the ribonuclease L (RNAse L) protein is known to be an important factor for antiviral response activation. This single nucleotide polymorphism (SNP) in RNAse L has been shown to block viral replication by degrading single stranded viral RNA and it is strongly implicated in the prevention of infection *in vivo* (Urisman et al., 2006). However, the experts believed that RNAse L SNP R462Q genetic susceptibility was not an important factor for the relative risk of XMRV infection (eg., being RR, RQ or QQ genotype for RNAse L at position 462 has little impact on infectivity).

Latency of XMRV was also considered to be an important risk factor. The experts believed that not all XMRV infections may result in persistent infections (transient infections may occur) and there was a need for nucleic acid testing detection in blood and plasma to include persistent viremia in chronically infected individuals. Experts believed that the majority of individuals infected with XMRV longer than three months (chronic infections) would have detectable antibodies and have detectable nucleic acids by nucleic acid testing (NAT) of their blood. The experts believed that the vast majority of individuals infected with XMRV were likely asymptomatic.

6. Routes of transmission for XMRV

To assess XMRV transmission the experts were asked to rank the risks associated with 12 potential routes of XMRV transmission using the method of pairwise comparisons (Macutkiewicz, 2008). With this approach, experts were presented with 12C2 = 66 pairs of routes, and asked to indicate which of the two routes being compared they considered to be riskier. In the pairwise comparison exercise, a draft list of XMRV transmission risk routes was presented to the expert group. The list was modified and agreed upon by the experts

after discussing the issues involved, and reaching group consensus on the definitions and related information pertinent to each of the transmission routes. The list of 12 potential routes of XMRV transmission is given in Tables 1 and 2.

The expert group's risk rankings associated with XMRV transmission were determined using the Probabilistic Inversion (PI) modeling option in UNIBALANCE (© TU Delft, available from: http://risk2.ewi.tudelft.nl/courses). This method produces a mean score for each of the transmission routes rated by the experts, with the standard deviation of the mean score providing a measure of uncertainty in expert opinion. The mean scores are rescaled to be between zero and one, so that the highest score is one (representing the route of transmission seen as most risky by the experts) and the lowest score is zero (the least risky transmission route).

The method is sophisticated enough to allow for both ties in ranking individual pairs of transmission routes being compared and inconsistencies in rankings across risk pairs. In addition to producing an overall ranking of the routes of transmission, the method provides a statistical test of the null hypothesis that a given expert is responding at random (in which case the results for that expert would be excluded from the analysis). The method also provides a test for inconsistency in response for a given expert. Finally, an overall coefficient of agreement ranging between 0 (complete disagreement) and 1 (complete agreement) among the experts is produced. Tests of inconsistency demonstrated that one of the experts appeared to give pairwise preferences randomly; this expert's responses were filtered out leaving 13 experts for the analysis.[4]

The unnormalized mean scores and associated standard deviations for the 12 potential routes of transmission of XMRV are given in Table 1. The normalized scores, anchored at zero (lowest perceived risk) and one (highest perceived risk), are shown in Table 2.

Rank	Transmission Route	Score	Standard deviation
1	Non-leukoreduced packed red blood cells	0.6982	0.2344
2	Solid organs	0.6915	0.2654
3	Bone marrow transplant	0.6655	0.2057
4	Hematopoietic stem cell transplant	0.6329	0.2454
5	Plasma transfusion	0.6113	0.2409
6	Leukoreduced packed red blood cells	0.5036	0.2488
7	Semen	0.4966	0.2537
8	Islet cells	0.3742	0.1963
9	Human derived urinary products	0.3431	0.2109
10	Tissues	0.2593	0.1885
11	Plasma derived clotting factor	0.2043	0.1926
12	Albumin	0.1991	0.1685

Table 1. Results of the pairwise comparison exercise unnormalized scores and standard deviation for 12 different XMRV transmission routes are shown.

---

[4] For more information and summary of the mathematical method used see, "Appendix 2. Description of the pairwise comparison by probabilistic inversion method" in: Tyshenko et al., (2011). For more information about the numerical algorithms used in this method see Macutkiewicz (2008).

The normalized scores indicate that, in relative terms, the ranking of the 12 routes from the most risky route to the least risky route were: non-leukoreduced packed red blood cells, solid organs, bone marrow transplant, hematopoietic stem cell transplant, plasma transfusion, leukoreduced packed red blood cells, semen, islet cells, human derived urinary products, tissue transplants, plasma derived clotting factor and albumin. Even though non-leukoreduced packed red blood cells were ranked as the highest risk the top four risks of non-leukoreduced packed RBC, solid organs, bone marrow transplant and hematopoietic stem cell transplants showed a non-significant difference. The four lowest perceived risks of XMRV transmission were human derived urinary products, tissues, plasma derived clotting factors and albumin. The lower values reflected the experts' views that processing clearance factors and product treatments likely reduce XMRV. Tissue was cited as being a low risk due to the lack of vasculature which would carry much less risk as compared to blood or solid organs. Experts believed albumin also possessed the smallest standard deviation which can be interpreted as having the least uncertainty in its ranking.

Relative Ranking	Transmission Route	Score (normalized)
1	Non-leukoreduced packed RBC	1
2	Solid organs	0.9867
3	Bone marrow transplant	0.9344
4	Hematopoietic stem cell transplant	0.8691
5	Plasma transfusion	0.8259
6	Leukoreduced packed RBC	0.6102
7	Semen	0.5962
8	Islet cells	0.3508
9	Human derived urinary products	0.2885
10	Tissue	0.1206
11	Plasma derived clotting factor	0.0104
12	Albumin	0

Table 2. Results of the pairwise comparison exercise showing the group's normalized scores ranked between 0 and 1 according to relative risk for 12 different XMRV transmission routes.

Overall, the experts considered 12 different routes of transmission, and all were deemed to be either medium to low for their probability to transmit XMRV infectivity. Transmission through blood and blood products split experts into two groups reflecting the uncertainty of XMRV virus infection as to whether it was cell or plasma based. Experts indicated the number of copies of virus in plasma could be variable depending on which stage of infection the donor is at. Regardless, the experts believed non-leukoreduced packed red blood cells, solid organs (due to their vasculature), bone marrow transplants, hematopoietic stem cell transplants, plasma transfusion and leukoreduced packed red blood cells would present the most likely transmission routes for XMRV (> 0.5). Other routes (< 0.5) including: semen, islet cells, human derived urinary products, plasma derived clotting factor, tissues and albumin would be the least likely to transmit XMRV given the list presented.

Experts ascribed low values to urine-derived pharmaceuticals (donor pool contaminated by 1 positive individual) due to low numbers of viral particles, large batches (10,000 in a donor pool) and clearance factor during purification. Experts discussed other routes of XMRV transmission not given on the pairwise comparison list and agreed transmission through breast milk and *in vitro* fertilization (IVF) techniques would be low. Similarly, fluids and cells free of white blood cells or plasma were also deemed to be low risk for XMRV transmission. Organs were seen as being riskier than tissues due to vascular systems and immunosuppression of the host. The individual discussions of other XMRV transmission routes correlated well with the results of the more structured pairwise comparison exercise that the expert group completed.

7. Risk mitigation of XMRV

Previous examples of Health Canada's regulatory actions based on a precautionary approach included actions to mitigate the risk of variant Creutzfeldt-Jakob disease (vCJD) and simian foamy virus (SFV) transmission. In both cases donor screening was added to defer those individuals with the highest exposure risk. For vCJD those with travel or residency history in high risk areas (United Kingdom and France) were deferred. The deferral took into account effects on the blood supply balanced against maximal risk reduction. As a result, the risk to transfusion recipients for vCJD was greatly reduced (Wilson et al., 2003). Similarly, donor screening for individuals at risk of exposure to SFV was implemented. Donor demographic screens balanced maximal risk reduction against the impacts to the blood supply. The risk of introduction of a retrovirus with the possibility of developing pathogenicity over time with spread in human hosts by transfusion has been reduced by precautionary actions.[5]

Considering XMRV, there were several risk mitigation options under consideration including: 1) Keeping the status quo, with further research, 2) Education and self-deferral, 3) Deferring donors presumed at risk for carrying XMRV, 4) Testing of donors for XMRV (not currently possible at the time of the expert meeting), and 5) Pathogen reduction strategies (a possible strategy for some components).

In Canada as of March 2010, individuals donating blood at Canadian Blood Services with a diagnosis of CFS were indefinitely deferred from blood donation. Prostate cancer patients are permanently deferred from blood donation, and donations from individuals, who were diagnosed with cancer post-donation, are retrieved, quarantined and destroyed.

Leukoreduction has been a useful risk mitigation strategy applied to other blood-borne pathogens. A Directive on Universal Leukoreduction was issued by Health Canada in 1998 specifying that in Canada that all cellular blood components must be filtered to remove white blood cells (WBCs) and residual WBC levels must be less than 5×10^6 cells per component (Health Canada, 1998). This action which is already in place in Canada was expected to have some protective effect for XMRV knowing that it was mostly associated with WBCs. Further validation studies would be needed to demonstrate the effectiveness of leukoreduction in preventing XMRV transmission through transfusion. The virus could still

---

[5] For donor deferral of SFV in Canada see: http://www.hc-sc.gc.ca/dhp-mps/brgtherap/activit/fs-fi/fact_simian_foamy_virus_spumeux_simien_feuillet-eng.php

be transmitted through plasma but data was lacking concerning titres necessary for infection.

The experts estimated a central value of 3 log reductions by leukoreduction but gave a consensus range that spanned 7 logs suggesting some uncertainty about the degree of clearance. Some experts highlighted the fact that whether the virus was cell-associated or from plasma viremia was an important consideration when determining the effectiveness of leukoreduction since this method likely would not reduce virus load found in plasma. When asked, the experts indicated that they had considered both cell-associated and plasma-associated virus when answering this question.

Experts believed plasma derivatives would have higher log reductions due to additional treatments that increased product purity. A log reduction of nearly 7 orders of magnitude by sterilization of medical devices was estimated by the expert group likely due to the heat treatment during standard autoclaving which would denature RNA viruses.

Even though leukoreduction is already in place in Canada, which would significantly reduce XMRV, the deferral of CFS patients was implemented as a precautionary measure. A similar measure was implemented in some other countries (for example, Australia and the United Kingdom).[6]

8. Disease relationships (causal and non-causal)

The probability of XMRV and its causal linkage to prostate cancer resulted in a lack of consensus from the expert group. The heterogeneity of prostate cancers was not considered. While XMRV nucleic acid and protein had not been isolated from the blood of prostate cancer patients it was found in about 1% of stromal cells, predominantly fibroblasts and hematopoietic elements in regions adjacent to the carcinoma (Urisman et al., 2006). Similarly, the experts indicated a low probability of association between XMRV and CFS but this was also associated with significant uncertainty. At the time of the meeting with high uncertainty surrounding XMRV as a potential emerging pathogen, the experts were very careful when interpreting and extrapolating the available research data. They acknowledged that the identification of XMRV RNA sequences in patient specimens by PCR assays was reported in the literature but it did not establish that infections had occurred and it did not prove disease causation or association with specific diseases. At this meeting some experts speculated about the possibility of sample contamination suggesting it may have occurred leading to contradictory cohort data. One important question that the experts agreed required more research was whether XMRV infection was a causal factor in the etiology of some CFS and prostate cancer cases or whether XMRV was merely a "passenger virus" identified in some healthy individuals and a subset of immunocompromised patient groups (e.g., CFS patients). Overall, the experts agreed that given the current understanding of XMRV that there was a very low probability that XMRV was implicated in disease etiology.

---

[6] For the Australian Red Cross Blood Service CFS deferral notice, see:
http://www.donateblood.com.au/media-centre/latest-national-news/blood-service-updates-cfs-donor-policy; for the United Kingdom National Health Service deferral notice see:
http://www.nhsbt.nhs.uk/news/2010/newsrelease071010.html.

9. Conclusions

The Krever Commission report (1997) acknowledged the benefits of using a reputable scientific evidence base when establishing public health policy to address any new emerging blood-borne pathogen in Canada. Krever concluded that, for emerging threats where there has been insufficient time to develop an adequate amount of scientific data to guide policy creation, such as those which periodically affect the blood supply, it is important to make the best possible effort to support policy making by means of qualitative and quantitative risk assessments in a timely manner (Krever, 1997; Wilson, 2007). Authorities should act even if there is only a theoretical risk of harm; and given the potential risk of an emerging pathogen regulators must err on the side of caution. Early on, in the absence of clear evidence, a precautionary approach for blood and CTO safety should be applied. Thus, the overarching guiding policy for any emerging pathogen should be the use of a precautionary approach.

As a result, Canadian regulators have adopted precautionary approaches in response to emerging and low probability but high consequence risk issues. The emergence of XMRV as a potential pathogen transmissible by blood resulted in a proactive and rapid response by Canadian risk managers and blood providers. In Canada a precautionary CFS blood deferral policy was put in place a few months prior to the expert meeting but at the time of the expert meeting the situation for CTO transplants remained an issue with uncertainty.

Risk management of emerging infectious retroviruses, such as XMRV, requires obtaining the needed contextual information, evidence (prevalence and incidence rates) and analyzing risk factors. Resources drawn upon include consultation with domestic experts, reviewing published and grey literature, awareness of global experience including accessing international expert opinion, utilizing available surveillance data, developing risk modeling with available data and considering other population health considerations.

During the 2009-2010 time period when high uncertainty existed surrounding XMRV the meeting helped to quantify knowledge gaps through the use of expert opinion. There was no direct evidence to support XMRV transmission through blood or CTOs; however, its detection in plasma and PBMCs of individuals in some studies suggested that these routes of transmission may be possible, and could significantly increase the risk to CTO recipients of exposure to this virus. The risks posed by XMRV to blood recipients would depend on its ability to cause diseases in humans, and the magnitude of this risk depends on whether only a subset of recipients (e.g., immunocompromised individuals) are susceptible to XMRV infection and disease or not. The route of transmission for any emerging blood-borne pathogen is dependent on the characteristics of the virus and the tissues affected.

The general population could potentially be exposed to a new emerging pathogen such as XMRV by various modes including vertical or horizontal transmission, as well as transmission through transfusion and transplantation. The detection of XMRV in plasma and PBMCs of infected individuals (Lombardi et al., 2009; Lo et al., 2010) suggested that there may be the possibility of a blood-borne transmission during transfusion. It was of concern that XMRV as an emerging pathogen might be transmitted through other body

fluids such as semen and breast milk although there was no direct evidence to support these as routes of infection. The expert pairwise comparison exercise confirmed that there was good agreement that these other routes were a low risk probability for XMRV transmission.

Regulation for CTOs used for transplantation in Canada, came into effect in early 1999. The regulations minimize the potential health risks to Canadian recipients of human CTOs by providing safety and processing requirements including: donor screening, donor testing, sample collection and retrieval, preservation, packaging, labelling, and quarantine), storage, record keeping, distribution, importation, error, accident and adverse reaction investigation and reporting.

At the time of the expert meeting the lack of scientific consensus regarding the association of XMRV with CFS in available published studies complicated the response for many countries and often polarized the experts from reaching consensus on XMRV issues under discussion at our meeting. Initially, contradictory reports were believed to be due to variation in the diagnosis of CFS patients, methodological variability between reported studies (use of PCR versus immunochemistry) and the use of differing and non-standardized samples or reagents. Later the contradictory reports were largely explained due to sample, tissue cell line and diagnostic kit contamination by mouse nucleic acids or VP62 plasmid.

Convening an expert group and soliciting the best available unbiased advice on uncertainty gaps provides a measure of due diligence and attentiveness to virtual surveillance for an emerging CTO and blood risk. Experts indicated that for any emerging pathogen further studies would be needed to address the gaps in information in order to appropriately assess the risk of transfusion transmitted infections. Generically, this would require three main activities: First, the development of standardized, well validated tests and methods to determine the prevalence of the pathogen in the general population and in blood donors. Secondly, research investigating its mode of transmission and different routes of transmission are important to initiate; and thirdly, further research and evidence confirming the pathogen's role in human disease (if it is not already established) is required.

As a case study XMRV exemplified an emerging potential blood-borne pathogen and how Canadian decision-makers implemented a precautionary approach. At the time there was significant uncertainty over the identified knowledge gaps discussed by the experts. At the time of the meeting the experts suggested that establishing the prevalence in the adult general population and in at risk groups within a Canadian context were key experiments to consider. This applies to XMRV or other emerging pathogens that may affect blood supplies.

We know now that the initial linkage of XMRV to CFS may have been due to sample contamination and false positives. At the time of these discussions the experts correctly believed that the uncertainty over XMRV would be resolved within the next two years from ongoing international research and publication of peer reviewed data. Virtual surveillance along with ongoing international expert consultation is necessary and vitally important to keep up-to-date about emerging blood-borne pathogens.

Finally, we note that the experts and decision-makers dealing with XMRV as an emerging threat to blood safety employed precautionary actions very early on. According to the Krever report every effort should be made to protect the blood supply, cells, tissues and organs from emerging blood-borne pathogens even if the risk remains largely unknown or a causal association with a disease state has not been established (Krever, 1997). XMRV turned out to be a contaminant; however, convening Canadian and international experts to discuss issues surrounding XMRV risk showed that, in Canada, when faced with uncertainty the use of precaution and mobilization of experts as discussed by Krever (1997) was put into full effect. These early interventions allowed for proactive management of this potential emerging blood borne threat.

10. Acknowledgements

11. References

Andonov A. 2010. "Screening for X/P-MLV Related Virus Sequences in Healthy Controls and Patients with Multiple Blood Transfusions". Public Health Agency of Canada, National Microbiology Laboratory, Ottawa, Canada. September 27, personal communication.

Aspinall W. 2010. A route to more tractable expert advice. Nature. 463(7279):294-5.

Bacich DJ, Sobek KM, Cummings JL, Atwood AA, and O'Keefe DS. 2011. False negative results from using common PCR reagents. BMC Res Notes. 4(1):457.

Carlowe J. 2010. Chronic fatigue syndrome is not caused by XMRV virus, study shows. BMJ. 341:c7358.

Carruthers BM, Jain AK, De Meirleir KL, Peterson DL, Klimas NG, Lerner AM, Bested AC, Flor-Henry P, Joshi P, Powles ACP, Sherkey JA, and van de Sande MI. 2003. Myalgic encephalomyelitis/chronic fatigue syndrome: clinical working case definition, diagnostic and treatment protocols. Journal of Chronic Fatigue Syndrome. 11:7–116.

Cohen J. 2011. More Negative Data for Link Between Mouse Virus and Human Disease. 331: 1253-54.

Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, and Komaroff AL. 1994. The chronic fatigue syndrome: a comprehensive approach to its definition and study. Ann Intern Med. 121:953-9.

Groom HC, Boucherit VC, Makinson K, Randal E, Baptista S, Hagan S, Gow JW, Mattes FM, Breuer J, Kerr JR, Stoye JP, and Bishop KN. 2010. Absence of xenotropic murine

leukemia virus-related virus in UK patients with chronic fatigue syndrome. Retrovirology. 7:10.

Health Canada. 1998. Directive D98-01: Implementation of Prestorage Leukoreduction of Cellular Blood Components, Nov. 2, 1998. Available at: http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/brgtherap/leukoreduction-deleucocytation-eng.pdf.

Holmes GP, Kaplan JE, Gantz NM, Komaroff AL, Schonberger LB, Straus SE, Jones JF, Dobois RE, Cunningham-Rundles C, Pahwa S, Tosato G, Zegans LS, Purtilo DT, Brown N, Schooley RT, and Brus I. 1988. Chronic fatigue syndrome: a working case definition. Ann Intern Med. 108:387-9.

Hong P, Li J, and Li Y. 2010. Failure to detect xenotropic murine leukemia virus-related virus in Chinese patients with chronic fatigue syndrome. Virol J. 7:224.

Hue S, Gray ER, Gall A, Katzourakis A, Tan CP, Houldcroft CJ, McLaren S, Pillay D, Futreal A, Garson JA, Pybus OG, Kellam P, and Towers GJ. 2010. Disease-associated XMRV sequences are consistent with laboratory contamination. Retrovirology. 7:111.

Kaiser J. 2011. Chronic fatigue syndrome. Studies point to possible contamination in XMRV findings. Science. 331(6013):17.

Knox K, Carrigan D, Simmons G, Teque F, Zhou Y, Hackett J Jr, Qiu X, Luk KC, Schochetman G, Knox A, Kogelnik AM, and Levy JA. 2011. No evidence of murine-like gammaretroviruses in CFS patients previously identified as XMRV-infected. Science. 333(6038):94-7.

Krever H. 1997. Volume 3: Chapter 40, The Blood System of the Future. Commission of Inquiry on the Blood System in Canada, Krever Commission. Canada, Public Works and Government Services Canada, Ottawa, Ontario.

Lo S-C, Pripuzova N, Bingjie L, Komaroff AL, Hung G-C, Wang R, and Alter H. 2010. Detection of MLV-related gag gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. Proc Natl Acad Sci U S A. 107:15874-9.

Lombardi VC, Ruscetti FW, Das Gupta J, Pfost MA, Hagen KS, Peterson DL, Ruscetti SK, Bagni RK, Petrow-Sadowski C, Gold B, Dean M, Silverman RH, and Mikovits JA. 2009. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science. 326:585-9.

Macutkiewicz M. 2008. Paired comparisons and group preference. Master of Science Thesis, Unpublished. Applied Mathematics Risk and Environmental Modeling, TU Delft; 92pp.

Oakes B, Tai AK, Cingoz O, Henefield MH, Levine S, Coffin JM, and Huber BT. 2010. Contamination of human DNA samples with mouse DNA can lead to false detection of XMRV-like sequences. Retrovirology. 7:109.

Paprotka T, Delviks-Frankenberry KA, Cingöz O, Martinez A, Kung HJ, Tepper CG, Hu WS, Fivash MJ Jr, Coffin JM, and Pathak VK. 2011. Recombinant origin of the retrovirus XMRV. Science. 333(6038):97-101.

Reeves WC, Wagner D, Nisenbaum R, Jones JF, Gurbaxani B, Solomon L, Papanicolaou DA, Unger ER, Vernon SD, and Heim C. 2005. Chronic fatigue syndrome-a clinically empirical approach to its definition and study. BMC Med. 3:19.

Robinson MJ, Erlwein OW, Kaye S, Weber J, Cingoz O, Patel A,Walker MM, Kim W-J, Uiprasertkul M, Coffin JM and McClure MO. 2010. Mouse DNA contamination in human tissue tested for XMRV. Retrovirology. 7:108.

Sato E, Furuta RA, and Miyazawa T. 2010. An endogenous murine leukemia viral genome contaminant in a commercial RTPCR Kit is amplified using standard primers for XMRV. Retrovirology. 7:110.

Schlaberg R, Choe DJ, Brown KR, Thaker HM, and Singh IR. 2009. XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. Proceedings of the National Academy of Sciences, U S A. 106:16351-16356.

Sharpe MC, Archard LC, Banatvala JE, Borysiewica LK, Clare AW, David A, Edwards RH, Hawton KE, Lambert HP, and Lane RJ. 1991. A report - chronic fatigue syndrome: guidelines for research. J Royal Soc Med. 84:118-21.

Silverman RH, Das Gupta J, Lombardi VC, Ruscetti FW, Pfost MA, Hagen KS, Peterson DL, Ruscetti SK, Bagni RK, Petrow-Sadowski C, Gold B, Dean M, Mikovits JA. 2011. Partial retraction. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science. 334(6053):176.

Smith RA. 2010. Contamination of clinical specimens with MLV-encoding nucleic acids: implications for XMRV and other candidate human retroviruses. Retrovirology. 7:112.

Switzer WM, Jia H, Hohn O, Zheng H, Tang S, Shankar A, Bannert N, Simmons G, Hendry RM, Falkenberg VR, Reeves WC, and Heneine W. 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.

Tuke PW, Tettmar KI, Tamuri A, Stoye JP, and Tedder RS. 2011. PCR master mixes harbour murine DNA sequences. Caveat emptor! PLoS One. 6(5):e19953.

Tyshenko MG, ElSaadany S, Oraby T, Darshan S, Aspinall W, Cooke R, Catford A and Krewski D. 2011. Expert Elicitation for the Judgment of Prion Disease Risk Uncertainties, Journal of Toxicology and Environmental Health, Part A. 74(2): 261-285.

Urisman A, Molinaro RJ, Fischer N, Plummer SJ, Casey G, Klein EA, Malathi K, Magi-Galluzzi C, Tubbs RR, Ganem D, Silverman RH, and DeRisi JL. 2006. Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathogen. 2(3):e25.

van Kuppeveld FJ, de Jong AS, Lanke KH, Verhaegh GW, Melchers WJ, Swanink CM, Bleijenberg G, Netea MG, Galama JM, and van der Meer JW. 2010. Prevalence of xenotropic murine leukemia virus-related virus in patients with chronic fatigue syndrome in the Netherlands: retrospective analysis of samples from an established cohort. Br Med J. 340.

Wilson K, Wilson M, Hébert PC, and Graham I. 2003. The application of the precautionary principle to the blood system: the Canadian blood system's vCJD donor deferral policy. Transfus Med Rev. 17(2): 89-94.

Wilson K. 2007. The Krever Commission-10 years later. CMAJ. 177: 1387-1389.

Yang J, Battacharya P, Singhal R, and Kandel1 ES. 2011. Xenotropic murine leukemia virus-related virus (XMRV) in prostate cancer cells likely represents a laboratory artifact Oncotarget 2011. 2: 358-362.
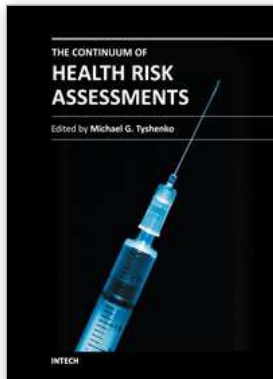
The Continuum of Health Risk Assessments

Edited by Dr. Michael G. Tyshenko

This book presents a collection of health risk assessments for known and emerging hazards that span a continuum. Case studies for existing health risks include psychoactive drug usage in delivery truck drivers and using look-back risk assessment for accidental syringe re-use in healthcare settings. Case studies for emerging risks include precautionary actions to safeguard blood supplies; nanoparticle deposition in the lung; and the epistemic issues surrounding genetically modified organism risk assessments. The final section of the book deals with advancing health risk assessment analyses through a post-genomics lens and provides case studies on personalized genomics, new data analyses and improving in silico models for risk assessment. These case studies provide much insight into the ongoing evolution of health risk assessments.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Michael G. Tyshenko, Susie ElSaadany, Tamer Oraby, Marian Laderoute, Jun Wu, Willy Aspinall, Daniel Krewski and Peter R. Ganz (2012). Xenotropic Murine Leukemia Virus-Related Virus as a Case Study: Using a Precautionary Risk Management Approach for Emerging Blood-Borne Pathogens in Canada, The Continuum of Health Risk Assessments, Dr. Michael G. Tyshenko (Ed.), ISBN: 980-953-307-582-7, InTech, Available from: http://www.intechopen.com/books/the-continuum-of-health-risk-assessments/xenotropic-murine-leukemia-virus-related-virus-as-a-case-study-using-a-precautionary-risk-managemen

INTECH
open science | open minds