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TECHNICAL REPORT

Risk assessment on xenotropic murine leukemia virus-related virus (XMRV) and its implications for blood donation

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Risk assessment on xenotropic murine leukemia virus-related virus (XMRV) and its implications for blood donation

Ad hoc expert panel on XMRV implications for blood donation

ECDC internal review group:

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External experts:

No external experts were involved in the production of this risk assessment.

The content and conclusions of this risk assessment were valid when sent to the European Commission on 22 July 2011.

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Contents

Abbreviations

AABB	former American Association of Blood Banks
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
CE	Conformité Européenne/European Conformity.
CFS	Chronic fatigue syndrome
CSF	Cerebral spinal fluid
DG SANCO	European Commission's Directorate General for Health and Consumers
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
env	Envelope
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridisation
gag	Group-specific antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HTLV	Human T-lymphotropic virus
IAP	Intracisternal A-type particle
IFA	Indirect fluorescent antibody
IFC	Immunofluorescent cell
ME	Myalgic encephalomyelitis
MLV	Murine leukemia virus
NIAID	National Institute of Allergy and Infectious Diseases
NZB	New Zealand black retrovirus
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
pol	Polymerase
RNase L	Ribonuclease L
RT-PCR	Reverse transcriptase polymerase chain reaction
SFFV	Spleen focus-forming virus
SV-40	Simian virus 40
VIPDx	Viral Immune Pathology Diagnostics
WB	Western blot
WPI	Whittemore Peterson Institute
XMRV	Xenotropic murine leukemia virus-related virus

Executive summary

Introduction

ECDC plays an important role in providing its stakeholders with scientific advice when requested. On 23 September 2010 ECDC received such a request from the European Commission's DG SANCO (Directorate C – Public Health and Risk Assessment).

ECDC was asked:

- to assess the epidemiological profile of XMRV (Xenotropic murine leukemia virus-related virus);
- to assess the scientific evidence of the link between chronic fatigue syndrome (CFS) and the presence of XMRV in the blood and transmission of XMRV via blood donation;
- to advise the Commission on the possible value of introducing deferral criteria and/or testing requirements in the EU.

Methodology

In accordance with its internal procedures for providing scientific advice, ECDC addressed the question by setting up an ad hoc group of internal experts. External experts were not consulted as ECDC had personnel in house with the relevant retroviral specialist expertise. Due to the novelty of the field it was possible to conduct a complete and systematic review of the entire published evidence base. This risk assessment therefore constitutes a complete assessment of the public knowledge currently available in the field. Evidence-based public health criteria were applied to assess the evidence for validity, generalisability and strength of documentation. In addition, the body of evidence was examined against Koch's postulates and the Bradford Hill criteria of causation.

Results and conclusions

The review of the literature showed that, based on an objective assessment of the knowledge available, a causal link between XMRV and CFS cannot be established. Recent work suggests that the original observation of such a putative association is more likely to be a laboratory artefact, caused by contamination of the cell cultures or PCR reagents used to investigate CFS patient material for evidence of XMRV infection. The Editor-in-Chief of *Science* magazine, the publisher of the original study linking XMRV and CFS, has taken the extraordinary step of requesting that the authors retract the study, which they have declined to do.

Examination of the evidence failed to convincingly support a causal link between XMRV and CFS on the basis of multiple criteria. From this perspective, an assessment of the virus' epidemiology among human populations is neither relevant nor possible.

ECDC would suggest the following for the Commission's consideration:

Donor screening

Implementation of blood donor screening on an EU-wide scale is not recommended at this time, as there is no published evidence of these viruses being transmitted via transfusion and no consistent proof of association with disease. Existing evidence strongly suggests that the original observations may be due to laboratory artefacts and that validated assays that detect infected individuals without implicating non-infected individuals have not yet been developed.

Donor deferral

Deferrals of blood donors with a history of CFS have been implemented in several countries as a precautionary measure until more data have been published. However, a critical examination of the existing evidence does not support this approach to address known risk as there has been no study suggesting transfusion transmission. If implemented, the justification would be applying an approach based on the precautionary principle alone. This measure could be re-examined after the conclusions of ongoing studies have been obtained, possibly already the end of 2011. At this time, the situation could also be monitored as the evidence does not suggest an immediate risk of blood-borne transmission.

Request from the European Commission

On 23 September 2010 the Director of ECDC received a request from the European Commission's DG SANCO (Directorate C – Public Health and Risk Assessment) to assess the possible implications of the Xenotropic murine leukemia virus-related virus (XMRV) on blood donation.

ECDC was asked, in particular, to assess (1) the epidemiological profile of XRMV, (2) scientific evidence of the link between chronic fatigue syndrome and the presence of XMRV in the blood and transmission via blood donation, and (3) to advise the Commission on the possible value and need of introducing deferral criteria and/or testing requirements in the EU.

The issue was discussed at the meeting of the competent authorities for blood in April 2010. As a precautionary measure countries such as Canada and New Zealand have introduced deferral of donors with a medical history of chronic fatigue syndrome, following scientific evidence that there may be a link between chronic fatigue syndrome and presence of XMRV in the blood.

Methods

Legal authority

According to the founding regulation of ECDC, Regulation (EC) No 851/2004[1] Art 9(2), 'the Centre may be requested by the Commission, the Member States, third countries and international organisations (in particular the WHO) to provide scientific or technical assistance in any field within its mission. Scientific and technical assistance provided by the Centre shall be based on evidence-based science and technology.'

ECDC shall:

- search for, collect, collate, evaluate and disseminate scientific data (Art 3(2)(a));
- provide scientific opinions and timely information (Art 3(2)(b),(c));
- exchange information, expertise and best practices (Art 3(2)(e)); and
- facilitate the development and implementation of joint actions (Art 3(2)(e)).

Evidence-based public health

Evidence-based decision-making in a public health setting involves carefully incorporating the best available scientific evidence from research and other reliable sources with consideration of values, perceived needs and measures in the given context. Evidence-based medicine is often defined as the integration of expertise, values, and the best available evidence into the decision-making process [1].

A public health decision might be rather complex, and needs to take into account several health determinants such as genetic factors, lifestyle, physical environment, socio-economic conditions, biological environment and health services at different levels [2].

Evidence-based methodologies

ECDC has performed this risk assessment using evidence-based methodologies in accordance with the following steps:

- Formulate questions,
- Search for evidence,
- Assess the evidence,
- Formulate an answer,
- Disseminate and implement,
- Evaluate.

Questions from the Commission

In the request, the European Commission asked ECDC to:

- assess the epidemiological profile of XMRV;
- assess the scientific evidence of the link between chronic fatigue syndrome and the presence of XMRV in the blood, and the transmission of XMRV via blood donation;
- advise the Commission on the possible value of introducing deferral criteria and/or testing requirements in the EU.

Search strategies

Reviews and original research articles were retrieved on several occasions from the PubMed and Embase bibliographic databases, most recently on 27 June 2011.

The search strategies submitted combined the concepts of XMRV and chronic fatigue syndrome (Annex 1). The concepts used in the search strategies applied the controlled vocabulary available in the bibliographic databases (i.e. MeSH and Emtree terms). The concepts were completed with multiple field search combinations by using natural vocabulary (i.e. keywords) according to the adequacy and number of results retrieved in each of the search strategies. The results were taken from all languages and limited to 2006 and onwards.

[1] Regulation (EC) No 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European centre for disease prevention and control. OJ L 142, 30.4.2004, p. 1.

Further searches were made in the Cochrane Library but the results retrieved were irrelevant, as were the results of a Scopus search to retrieve grey literature on the topic using the Scirus search engine.

A total of 157 abstracts were retrieved and read, from which 108 publications were selected for further reading. Some additional publications and conference abstracts were later sourced from the references. The conference abstracts were used to provide insight into possible upcoming publications, but not included in the evidence table.

The following sections are included in the evidence table (Annex 2):

- Bibliographic citation
- Study type, country, number and type of patients, clinical criteria
- Methodology and cell type or fluid tested
- Outcome and XMRV prevalence
- Strengths and limitations of study.

Assessment of the evidence

Validity. To assess the validity of a study is to evaluate whether the results of the study are trustworthy.

Generalisability (external validity). An assessment of external validity or generalisability involves evaluating whether the studies are transferrable to other settings or circumstances.

Grading of evidence according to strength of documentation. Applying an evidence-based approach involves drawing explicit conclusions and building on the best available evidence to give more weight to the highest quality studies employing the most robust methods. Nevertheless, studies with a less robust design can be judged according to their quality. A study can be high quality even if its design indicates that little weight can be given to the evidence.

References: Background and methods

Straus SE, Richardson WS, Glasziou P, Haynes RB. Evidence-Based Medicine. How to Practice and Teach EBM. Edinburgh: Churchill Livingstone; 2005.

Gray M. Evidence-based Health Care and Public Health: How to Make Decisions About Health Services and Public Health. 3rd ed. Edinburgh: Churchill Livingstone; 2008.

Current EU requirements for testing and donor deferral

Directive 2002/98/EC (amending Directive 2001/83/EC) and its implementing measures (2004/33/EC and 2005/62/EC) set out European minimum requirements for the donation, testing, processing, storage and distribution of human blood and blood components.

There is currently no requirement for XMRV testing within this regulation and neither is there a requirement to defer individuals that show clinical signs or symptoms of CFS or have a history of CFS.

Background

Chronic fatigue syndrome

In humans, chronic fatigue syndrome (CFS), also referred to as myalgic encephalomyelitis (ME), is a disease of unknown aetiology but suspected to involve viral infection and characterised by debilitating fatigue and other symptoms [1]. Although chronic inflammation is often found in patients suffering from CFS, no infectious or toxic agent has been clearly implicated in the disease, which is diagnosed largely by excluding other conditions that cause similar symptoms [1].

CFS is characterised by new onset of unexplained, persistent or recurrent fatigue, diffuse post-exertional malaise and/or fatigue, myalgia, sleep dysfunction, and neurological/cognitive impairment with immune, autonomic and/or neuroendocrine manifestations of six months duration or longer (three months in children). Symptoms are not caused by ongoing exertion, are not relieved by rest, and result in a substantial reduction of occupational, educational, social, or personal activities compared with previous levels. Co-morbid conditions, such as fibromyalgia syndrome and irritable bowel syndrome may overlap with CFS.

The most widely used CFS case definition for research follows the Fukuda criteria published by an international study group led by the Centers for Disease Control and Prevention (CDC) in 1994 [2,3]. This definition is based on clinical criteria in which patients are required to exhibit persistently disabling fatigue of at least six months' duration accompanied by at least four of a possible eight symptoms. The 2003 Canadian Clinical Consensus Criteria provide more specific symptom definitions, but were intended for application in clinical settings and have not been used broadly to define research cohorts [4]. Both definitions have been criticised for containing vaguely worded criteria that lack operational assessments for measuring and scoring the severity, duration and co-occurrence of symptoms. A modified tool based on the Canadian case definition and using operationally explicit, objective criteria for specific key symptoms has recently been proposed, but has not yet been validated [5]. Other definitions for CFS have been published and used by groups to select subjects whose samples have been tested for XMRV. These definitions include the first CFS definition by Holmes and colleagues [6], the Oxford criteria [7] and CDC empiric criteria [3]. However, there are still no established objective diagnostic standards or biomarkers for CFS.

Xenotropic murine leukemia virus-related virus

Half a century of studies on murine leukemia viruses (MLVs) and other gammaretroviruses has demonstrated no clear evidence of human infection or causal association between these viruses and any human disease. The first instances of human infection with gammaretroviruses was reported in 2006 when genome sequences from a previously undescribed gammaretrovirus were detected in a US cohort of men with localised prostate cancer undergoing radical prostatectomy [8]. The investigators proposed that these patients expressed a homozygous mutation (R462Q) of the antiviral enzyme ribonuclease (RNase) L, rendering them unusually susceptible to the oncogenic potential of the virus. This novel virus was named Xenotropic Murine Leukemia-Related Virus (XMRV).

XMRV is a member of the *Gammaretrovirus* genus of the *Orthoretrovirinae* subfamily of *Retroviridae* with high sequence similarity to endogenous MLVs [9]. Xenotropic MLVs can infect foreign cells, such as human cells, but do not re-infect murine cells. As the name implies, XMRV is believed to have originated in mice and is the first agent of its class to be identified in humans; it likely evolved as a result of a recombination event between polytropic and xenotropic MLV. Similar agents are found in a wide range of mammalian species and include the porcine endogenous retrovirus, the feline leukemia virus, the koala retrovirus and the gibbon ape leukemia virus, that can cause leukemia and other syndromes in their host species.

A recent study [10] suggests that XMRV may have originated through a recombination event of two proviruses in the human prostate tumour xenograft CWR22 between 1993 and 1996 while being grown in a mouse. These proviruses (PreXMRV-1 and PreXMRV-2) share 99.92% identity with XMRV over >3.2-kilobase stretches of their genomes, differing by only a single nucleotide. The authors of this study noted that the probability of an identical recombination event occurring independently is about one in one trillion, making it extremely unlikely that XMRV arose from another source. The prostate cancer cell line CWR22Rv1 (derived from CWR22) has been shown to include multiple integrated copies of replication-competent XRMV [11].

Gammaretroviruses are much simpler than the complex deltaviruses such as human T-lymphotropic virus (HTLV), or the complex lentiviruses such as human immunodeficiency virus (HIV). The XMRV genome includes *gag*, *pol*, and *env* genes but no accessory or regulatory genes [9].

The receptor for XMRV is XPR1, a cell-surface protein ubiquitously expressed in many human and animal cells. In vitro assays show that the virus can infect and replicate in various human, feral mouse, mink, monkey, and bovine cell lines [12]. A recent intravenous infection study [13] of adult Indian rhesus macaques (described in the 'Animal models' section below) shows that XMRV may have the capability to infect humans.

Scientific studies

XMRV and MLV in CFS patients

Observations of RNase L proteolysis in peripheral blood mononuclear cells (PBMCs) of patients with CFS [14, 15] and chronic immune system activation resembling a viral infection [1] prompted a search for XMRV in patients with this disorder. Details of the studies currently published are listed in Annex 2.

In October 2009, Lombardi et al. [16] reported finding XMRV in PBMC proviral DNA using PCR from about 67% (68 out of 101) of CFS patients, compared to only 3.6% (5 out of 218) of healthy persons. Secondary infections in tissue culture could be established from PBMCs, B- and T-cells and plasma of patients. Circulating antibodies against the XMRV envelope protein were detected in 18 patients with CFS, but not in the plasma of seven healthy controls. The viral genomes from three patients with chronic fatigue syndrome were also sequenced and found to be >99% identical to XMRV found in prostate cancer [8]. There was no association with the RNase L mutation in either group.

The Lombardi et al. study prompted a great deal of activity in the field. Four subsequent studies in the first half of 2010, two in the United Kingdom [17, 18], one in the Netherlands [19] and one in the United States [20] reported no evidence of XMRV infection in patients with CFS.

In August 2010, Lo et al. published a study [21] reporting an association between CFS and a different murine leukemia virus (MLV)-related virus. Using nested PCR assays, the authors found MLV-like virus *gag* gene sequences in 32 out of 37 (86.5%) blood samples of CFS patients, but only in 6.8% (3 out of 44) of the samples from healthy volunteer donors. The *gag* and *env* sequences from the CFS patients were more closely related to those of polytropic mouse endogenous retrovirus than to those of XMRVs.

The Lo et al. study was followed by an additional four studies on CFS patients in China [22], Germany [23] and the United States [24, 25], where no XMRV was found. Following these studies, Erlwein et al. retested the patient cohort from their previous study [17] using additional methodology, with negative results [26]. This was then followed by two other studies in Japan [27] and the United States [28], also reporting negative results.

Although at this point many studies had reported negative findings, a number of the studies had limitations, such as small control populations; patient and control samples collected at different times; investigators generally not blinded to sample identity; unknown limits for detection, reproducibility, and precision of assays; insufficient numbers of negative controls included, etc. No study included positive samples from the original 2009 patient cohort of Lombardi et al. However, in May 2011, two major studies from the United States were published that addressed these issues.

In the first of these studies, by Shin et al. [29], the authors collected blood from 100 CFS patients fulfilling both the CDC/Fukuda and the Canadian consensus criteria for diagnosis of ME/CFS and from 200 healthy volunteers in the Salt Lake City area. The blood samples were extensively analysed using molecular, serological, and viral growth assays, including assays used by Lombardi et al. and Lo et al. The authors also analysed samples from individuals who had participated in the Lombardi et al. study. Those samples were obtained by a third-party phlebotomy service that collected blood during home visits, de-identified the samples and sent them to the authors' lab. All samples were analysed in a blinded manner. No XRMV was found in any sample, regardless of methodology.

The second study, by Knox et al. [30], addresses concerns about case criteria used and how patients were selected by obtaining samples from Dr. Daniel Peterson, one of the physicians involved in the Lombardi et al. study, to eliminate these as confounding variables.

The Knox et al. study involved two groups of CFS patients, called P1 and P2. P1 included 41 CFS patients, 37 of whom had been tested for XMRV by the Whittemore Peterson Institute (WPI) – where Lombardi et al. are based – or its commercial laboratory, Viral Immune Pathology Diagnostics (VIPDx), using whole blood PCR, serum PCR or viral XMRV culture with PCR. In the Lombardi et al. study 26 of the 37 patients tested by WPI/VIPDx were XRMV-positive, the other 11 were negative. In the Knox et al. study, all 41 patients in P1 were tested for XMRV using nested PCR and all were negative. In addition, 19 of the 41 P1 patient samples had been drawn by the same phlebotomist, with one sample being sent to VIPDx and one to the authors' lab. A comparison of these results showed that VIPDx detected XMRV in 10 of 19 samples, while Knox et al. did not detect XMRV in any of them.

This finding led the investigators to collect blood samples from a second group of patients (P2) at the same medical practice. This group of 29 CFS patients included 26 patients who were selected because they had previously tested positive for XMRV at WPI or VIPDx. Nine people were included in both P1 and P2 and fresh samples were collected from all 29 subjects in P2. The team used a number of techniques that were based on the original Lombardi et al. and Lo et al. reports, including reverse transcription PCR (RT-PCR) and virus culture. None of the 29 samples were found to be XMRV or MLV positive using any of these methods.

XRMV in other non-CFS populations

Since the original finding of XMRV in 2006 [8], many other studies have looked for XMRV in prostate cancer patients, reporting both positive [31-37] and negative [27, 38-41] results. In February 2011, a study was published casting doubt on the positive findings, suggesting that the patient-derived positive results from earlier studies of XMRV in prostate cancer patients may be the result of PCR contamination originating from experimentally infected cell lines [42].

Other studies have tried to find XMRV in patients with HIV [43-48], multiple sclerosis [23, 49], hepatitis C [43], autism [50, 51], paediatric idiopathic disease [52], fibromyalgia [53, 54], amyotrophic lateral sclerosis [55], systemic lupus erythematosus [56] and chronic immunomodulatory conditions [24], all with negative results.

The only study in populations not including CFS and/or prostate cancer patients that has reported positive findings is from Germany, where investigators detected XMRV in respiratory secretions using PCR [57]. However, the finding of XMRV sequences in respiratory secretions does not prove that the virus can be transmitted by the respiratory route, as retroviruses are not known to spread by respiratory transmission.

Contamination

Since the publication of the Lombardi et al. and Lo et al. studies, several papers have been published challenging the validity of their findings and suggesting possible contamination sources for these studies. Four independent studies describe finding mouse DNA contamination in patient samples [29, 31, 38, 58], which could be amplified in PCR-based assays using XMRV primers [59, 60]. One of these studies traced the contamination to robotic equipment that had previously been used to extract DNA from XMRV-infected tissue culture cells several months earlier [29]. The other three studies were not able to trace the source of the contamination.

Four additional studies have found MLV sequences in commonly used brands of *taq* polymerases [29, 30, 60, 61], however not in the brand used in the Lombardi et al. or Lo et al. studies. Knox et al. [30] also performed an analysis that showed three of the XMRV sequences reported by Lombardi et al. were 98–100 % identical to the infectious molecular clone VP62, the specific sequence of XMRV cloned from prostate cancer tissue. They therefore suggested that the genetic conservation of the Lombardi et al. XMRV sequences could be explained by laboratory contamination of PCR assays with the VP62 clone which was used by WPI in its original experiments.

One study stated that sequences from two previously published XMRV integration sites which used patient-derived human prostate cancer cells were identical to those from experimentally infected cell lines used in the same laboratory [62].

These studies confirm the importance of carefully checking XMRV/MLV-related positive results for any evidence of contamination with mouse genetic materials, which are very common in most laboratories. However, they do not prove that the positive samples in the Lombardi et al. or Lo et al. studies actually were due to contamination, although this seems to be the most plausible explanation.

Animal models

Although little is known about XMRV infectivity, tissue tropism, in vivo reservoirs and persistence in humans, preliminary information has been obtained from an animal model [13, 63]. In these animal exposure studies, five rhesus macaques, inoculated intravenously with XMRV isolate VP62, developed transient, low-level viremia between Days 4 and 21. XMRV established a persistent, chronic, disseminated infection, with low transient viremia and provirus in blood lymphocytes during acute infection. Although undetectable in blood after about a month, XMRV viremia was reactivated at nine months, confirming the chronic nature of the infection. Furthermore, XMRV *gag* was generally detected in tissues and widely disseminated throughout the entire period of monitoring. The XMRV infection showed organ-specific cell tropism; CD4 T-cells in lymphoid organs and epithelial/interstitial cells in other organs, including the reproductive tract. In spite of the intravenous inoculation, extensive XMRV replication was noted in prostate during acute but not chronic infection, even though infected cells were still detectable using a Fluorescence In Situ Hybridization (FISH) test in prostate at five and nine months post infection. None of the animals showed any obvious clinical symptoms.

Ongoing studies

As the scientific data on this topic is conflicting, there are a multitude of studies on XMRV currently ongoing. Of special interest are two large studies that have been initiated in the United States, the first coordinated by Ian Lipkin at the National Institute of Allergy and Infectious Diseases (NIAID), and the second by the Blood XMRV Scientific Research Working Group (National Heart, Lung, and Blood Institute), to study any potential connection between XMRV/MLV and human health or risks to the blood supply.

The NIAID study involves fresh blood samples from 100 CFS patients and 100 similar, but healthy people. These have been equally divided so that they come from four different sites around the country, to provide geographic diversity. The samples will be processed, blinded and sent to the Food and Drug Administration (FDA), the CDC and the Whittemore Peterson Institute (which led the team that published the original *Science* paper), who will test them using each laboratory's own protocols. The results will be analysed and the code broken by Lipkin's group at Columbia's Center for Infection and Immunity. Results are expected by the end of 2011.

The Blood XMRV Scientific Research Working Group study aims to evaluate XMRV detection assays and is organised into the following four phases:

- Phase I: Analytical panels – evaluate performance of XMRV nucleic acid test assays
- Phase II: Pilot clinical studies – compare assays using whole blood versus PBMCs and evaluate timing of sample preparation
- Phase III: Clinical sensitivity/specificity panel – assesses assay performance on pedigreed clinical samples
- Phase IV: Blood donor clinical panel – makes initial estimate of XMRV nucleic acid prevalence in blood donors and initiates blood donor seroprevalence studies.

Initial studies on sample panels, constructed by spiking blood and plasma with a virus or infected cells containing an original isolate of XMRV from prostate cancer patients, have shown that those nucleic acid test systems evaluated to date appear to be sensitive and broadly comparable [64]. Subsequent data, reported but not published and using samples from CFS patients and pedigreed negative controls, have been less easy to interpret [65].

Two additional studies are currently being conducted by the American Red Cross [66].

- The first study will involve collecting donations from over 10 000 healthy people in six different geographic areas to look for evidence of XMRV or MLVs, either through the detection of antibodies or the presence of small amounts of viral RNA. The tests will be run by Gen-Probe and Abbott Laboratories, two companies that have been developing tests for XMRV and MLVs.
- The second study involves a linked donor-recipient blood repository maintained by the Red Cross. The group will be looking at 120 recipients who received blood from over 4 000 donors. Donors will be tested to see if they are positive for XMRV or MLVs and recipients will then be tested to see if there was transmission of the virus through transfusions.

Preliminary results and abstracts presented at the 1st International Workshop of Xenotropic Murine Leukemia Virus-Related Virus [67, 68] in September 2010, an XMRV conference in Oslo in November 2010 [69] and the 15th International Conference on Human Retroviruses: HTLV and Related Viruses, Belgium, in June 2011 [70] indicate that studies with positive findings of XRMV/MLV in CFS patients from the United States, United Kingdom and Norway may be published in the near future. As yet, however, no such studies have been published in peer-reviewed journals.

Editorial expression of concern

On 2 June 2011, the editor-in-chief of *Science* magazine published an editorial expression of concern [71], after having contacted the authors of the original study [16]. The editor-in-chief had asked the authors to voluntarily retract the paper 'in light of the growing number of research papers from independent investigators who have either failed to replicate your original finding that XMRV is associated with chronic fatigue syndrome and/or who have provided evidence that laboratory reagents are widely contaminated with the virus' [72].

The authors declined the request in a letter to *Science*, calling the action premature.

Epidemiological profile of XMRV

This section pertains to question 1) of the Commission query.

At present, information regarding the prevalence of XMRV in either patient populations or volunteer donor populations remains fragmentary and controversial. The identification of gammaretroviruses in volunteer donors [16] suggests that there may be asymptomatic chronic carriers. However, these findings may be result from the false-positive results of laboratory assays, laboratory contamination or cross-reaction to naturally-occurring but inactivated proviral sequences common in the human genome. Long-term persistence of MLV-like virus has been reported in some symptomatic patients for as long as 15 years [21], but the same caveat as above applies.

Therefore, it is neither possible nor relevant to objectively assess the epidemiology of XMRV as there is no certainty to the original observations associating this virus with human disease.

Assessment of the strength of evidence for a causal association between XMRV and CFS

For this risk-assessment it was possible to examine the entire body of evidence available in published studies concerning XMRV. This was possible because the field is new, XMRV having been described for the first time in 2006.

As regards the validity of the evidence for a causal association between XMRV and CFS, the conclusion is that there are serious methodological questions concerning the only two studies showing positive results for an association. One highly probable explanation of these results is the contamination of patient materials and/or reagents. Given that multiple parallel studies have produced negative results and independent repeat studies have been unable to replicate the original results, it is highly likely that there is no association.

The same conclusion is supported in relation to generalisability or external validity. The original observations of a possible link have not been transferable to other settings and attempts to replicate have not been able to confirm the original results. While a cloned virus has been shown to be replication-competent and able to establish an infection in a variety of cell types and lines and even in macaques, this alone does not constitute evidence of generalisability. Mouse and human genomes contain multiple copies of ancient retroviral provirus sequences, which may be 'repaired' in vitro to re-establish their replication competence.

Studies constituting the evidence base have revealed that the viral clone called XMRV may have been generated by a random recombination event during propagation of human cells in a mouse model which happened to restore replication competence. This clone may then have spread undetected to a number of cell lines in various research laboratories.

Undetected contamination by retroviruses (and other viruses) in cell culture is not an unusual or previously unknown phenomenon. It has even resulted in the publication of newly discovered viruses in reputable journals, with the source not being discovered until much later. One of the best known examples was the contamination of cell cultures at Dr Robert Gallo's laboratories in the United States with the LAV-1 strain of HIV-1, sent by Dr Luc Montagnier in France. This resulted in Gallo's research group essentially isolating the LAV strain and claiming it to be an independent strain for several decades [31–33]. Eventually an agreement was reached between the US and French investigators [34], but the contamination is believed to have influenced the decision by the Swedish Academy to award the 2008 Nobel Prize for Medicine for the original discovery of HIV-1 only to the French investigators [35].

Another similar event took place in 1986 when a supposedly new human retrovirus, HTLV-4, was described in a study published in *Science* [36]. Subsequent sequence analysis of the virus showed that the observation was false. In fact, a culture of simian virus (STLV-IIIAGM) had contaminated human cell cultures [37] in the laboratory.

The above examples make a strong case for the possibility that XMRV may be a similar question of mixed identity and contamination. If the scientific principle of parsimony (i.e. the principle that the simplest possible explanation most often is the correct one) is followed, this is the most likely explanation for the findings.

Another way of examining whether there is sufficient evidence to support causality between infection with a certain microbe and a disease/syndrome is to use one of the logical tools available for this purpose. These tools can approach the problem from both the microbiological and the epidemiological perspective. The oldest of them are Koch's postulates and the Bradford Hill criteria.

Koch's postulates

The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884 and refined and published by Koch in 1890. Koch applied the postulates to establish the aetiology of anthrax and tuberculosis, but they have been generalised to other diseases. In their original form they state that:

- The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
- The microorganism must be isolated from a diseased organism and grown in pure culture.
- The cultured microorganism should cause disease when introduced into a healthy organism.
- The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Koch's postulates have played an important role in microbiology, yet they have major limitations. Many microbes only cause disease in a limited number of individuals out of those infected or colonised. More recently, modern nucleic acid-based microbial detection methods have made Koch's original postulates even less relevant. Fredricks and Relman have suggested the following set of revised Koch's postulates for the twenty-first century:

- A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased, and not in those organs that lack pathology.
- Fewer, or no, copy numbers of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease.
- With resolution of disease, the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the opposite should occur.
- When sequence detection predates disease, or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship.
- The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms.
- Tissue-sequence correlates should be sought at the cellular level: efforts should be made to demonstrate specific in situ hybridisation of microbial sequence to areas of tissue pathology and to visible microorganisms or to areas where microorganisms are presumed to be located.
- These sequence-based forms of evidence for microbial causation should be reproducible.

The Bradford Hill criteria

While Koch's postulates stem from the microbiological and experimental/laboratory science field, epidemiological criteria have also been developed for the examination of epidemiological associations.

The Bradford Hill criteria, otherwise known as Hill's criteria for causation, are a group of minimal conditions necessary to provide adequate evidence of a causal relationship between an incidence and a consequence, established by the English epidemiologist Sir Austin Bradford Hill in 1965.

The list of the criteria is as follows:

- Strength of association (often measured by odds or risk ratio).
- Consistency: consistent findings observed on different occasions reinforce the likelihood of an effect.
- Specificity: causation is likely if a very specific population at a specific site and disease with no other likely explanation. The more specific an association between a factor and an effect is, the bigger the probability of a causal relationship.
- Temporal relationship (temporality): The effect has to occur after the cause.
- Biological gradient (dose-response relationship): Greater exposure should generally lead to greater incidence of the effect. However, in some cases, the mere presence of the factor can trigger the effect. In other cases, an inverse proportion is observed: greater exposure leads to lower incidence.
- Plausibility (biological plausibility): A plausible mechanism between cause and effect is helpful (but Hill noted that knowledge of the mechanism is limited by current knowledge).
- Coherence: coherence between epidemiological and laboratory findings increases the likelihood of an effect. However, Hill noted that '... lack of such [laboratory] evidence cannot nullify the epidemiological effect on associations'.
- Experiment (reversibility).
- Analogy (consideration of alternate explanations).

Critical examination of both Koch's postulates and the Bradford Hill criteria reveals that the evidence for a causal relationship between XMRV and CFS does not pass the test at this time, since major elements of the criteria are not fulfilled. Taken together causation cannot be verified.

Testing for XMRV

Several research grade assays for direct detection of XMRV and serologic detection of exposure to XMRV are being used in research laboratories.

- PCR assays (nested and real-time PCR) and FISH for direct detection of viral sequences.
- Serologic assays for detection of circulating antibodies against XMRV. These include flow cytometry, western blot (WB) and chemiluminescent immunoassays and enzyme-linked immunosorbent assay (ELISA) techniques.
- Immunohistochemical assays for direct detection of viral proteins.
- Cell culture assays used to detect an infectious virus.

It has been difficult to compare assay results from various laboratories due to differences in primer pair or antigen selection, assay reaction conditions, specimen type, and specimen preparation and storage conditions. Efforts to standardise these assays are well underway, and there is commercial interest in developing diagnostic nucleic acid testing and serologic assays for licensing. Further research is necessary before any of these tests can be used reliably, and validation of the tests will be critical for large-scale epidemiologic studies.

Currently there are no FDA-licensed diagnostic or blood donor-screening assays. No test has received the CE mark approval for marketing in the EU.

Possible implications for blood donation

Transfusion-mediated transmission of gammaretroviruses would theoretically be possible if the observations of XMRV presence in PBMCs and in plasma can be verified, and if viruses capable of infecting human cells in the laboratory can be found in human specimens [16]. Other pathogenic retroviruses (e.g. HIV and HTLV) are clearly transfusion-transmitted and in the rhesus macaque animal model [31], XMRV has been shown to infect lymphoid cells and to disseminate to other tissues. However, several studies have been unable to repeat the observation of the XMRV sequences present in patients with CFS and other populations. In addition, several recent studies have seriously implicated contamination (either by viral sequences, murine retrovirus cultures or murine proviral retrovirus containing mouse DNA) as the reason for the original observations of a link between XMRV, CFS and prostate cancer.

Actual transmission from transfusion has, however, not been documented in humans. At this stage, discussions concerning blood donation deferral for patients with chronic fatigue syndrome are based on the general principle that there might be an infectious aetiology, not on specific diagnostic testing.

Actions taken by countries and organisations worldwide

At present, no country is testing for XMRV or has banned donation of blood, organs or other tissues from individuals who test positive for XMRV. However, due to the possible risk of a retroviral cause for CFS, several countries have applied the precautionary principle and introduced indefinite deferrals for blood donation from patients diagnosed with CFS as an interim measure until more solid data has been published.

United States

In the United States, two workgroups were established after the publication of the original *Science* article to evaluate the potential for XMRV transmission by blood transfusion and to advise the transfusion medicine community on appropriate responses.

The Blood XMRV Scientific Research Working Group has been charged with providing data on three issues: defining the prevalence of XMRV/MLV infection in the US blood donor population; determining whether these viruses are transmissible by transfusion and, if such transmission occurs, defining its clinical significance. To date, the group has focused on defining the performance characteristics of the tests available in order to establish a standardised approach (see the 'Ongoing studies' section above). They have also provided a brief literature review of epidemiological data that fail to support a relationship between blood transfusion and prostate cancer or CFS [32].

In December 2009, the AABB (formerly the American Association of Blood Banks) established an inter-organisational task force consisting of blood collectors, government agencies and non-profit organisations dedicated to CFS research and policy, supplemented by scientific consultants. The task force was charged with reviewing the data available on XMRV; recommending action to assess and mitigate the risk of transmitting XMRV through blood and cellular therapy products, and advising AABB on how to inform donors, recipients, physicians, and the general public of the risk of XMRV transmission. In January 2011, AABB published an interim report in the journal *Transfusion* [33]. In brief, they suggested that there was insufficient scientific information to make recommendations relating to XMRV/MLV. However, they recommended that blood collecting organisations should actively discourage blood donation by those with a current or past medical diagnosis of CFS. Materials were provided to educate potential donors about the issues. This recommendation is consistent with existing recommendations that CFS patients should refrain from giving blood, largely for their own protection. However the recommendation also recognises the fact that many chronic infections have been potentially linked to CFS.

The American Red Cross and a number of independent blood centres have implemented the AABB recommendation and are monitoring response to these educational materials. The American Red Cross states [34] that it defers indefinitely anyone who reports a past or present diagnosis of CFS. They are also trying to contend with the possible existence of XMRV in the blood supply by conducting two simultaneous studies (described in 'Ongoing studies' above).

At a meeting of the FDA Blood Products Advisory Committee (BPAC) on 14 December 2010 [35], the Committee was asked to vote on the following question: Do the scientific data support asking donors about a medical history and/or diagnosis of CFS as a basis for indefinite deferral? Nine members voted yes and four voted no. The 9-4 vote

reflects opinion on the issue of whether to ask a question or use the AABB educational materials to elicit donor disclosure of past/present CFS diagnosis. All BPAC members have indicated that they agree with the indefinite deferral of CFS patients based on evidence indicating that this will promote donor and recipient safety. The FDA gives serious consideration to guidance from its advisory committees when making policy, although as yet no decision has been made.

Canada

In April 2010, Canadian Blood Services changed their policy for blood donors with a history or current diagnosis of CFS, deferring them from donating for two years [36].

New Zealand

The New Zealand Blood Services followed Canada's new guidelines, deferring people with a diagnosis of chronic fatigue syndrome from donating blood in New Zealand [37].

Australia

The Australian Red Cross announced in April 2010 that it would indefinitely defer donors with a history or current diagnosis of CFS, stating that this precaution was taken to protect blood recipients until more is known about XMRV [38]. The policy will be re-evaluated in two years.

United Kingdom

Since 1 November 2010, the United Kingdom has been permanently deferring donors with a past or current history of CFS [39]. As a precaution, donor selection guidelines were changed to protect the donor's safety by ensuring the condition is not made worse by donating blood, bringing donor selection guidelines for ME/CFS into line with other conditions where individuals are permanently excluded from blood donation to protect their own health.

Summary of findings

Retroviruses are an important group of pathogens in animals and humans that cause a variety of diseases, including cancers and chronic inflammatory conditions. Four human retroviruses have been discovered since the 1980s: HIV-1, HIV-2, HTLV-1 and HTLV-2.

The original study that identified a link between XMRV and CFS (Lombardi et al.) still remains unconfirmed and has been questioned with regard to the prevalence of in vivo XMRV infection and its causal disease relationship to CFS. Transmission of xenotropic murine leukemia viruses to humans is theoretically possible as human cells do express the XPR1 protein that is able to function as receptor for xenotropic and polytropic murine retroviruses. However, the fact that XMRV can infect lymphoid tissues, which is not contested, does not establish causality of CFS. The first step to determine a microbial agent's etiological role in relation to a disease is to establish an association of the infection with the disease in question. So far, efforts to assess the prevalence of XMRV in humans, including patients with CFS, prostate cancer and healthy individuals, have resulted in widely disparate conclusions. To date there is no conclusive evidence of a causative relationship between XMRV and any human disease.

The risk of transfusion-transmitted XMRV infection will depend on several viral biologic and epidemiologic factors including the possible prevalence, if any, of XMRV infection in the donor population; the length of the asymptomatic blood-borne period; viral load in asymptomatic donors, and the immune status of transfusion recipients. The well-documented examples of transmissibility through blood which exist for other human retroviruses, such as HIV and HTLV, certainly seem to indicate that XMRV may also be transmitted by blood. However, as the validity of the observations relating to human infection is highly questionable, such possibilities are chiefly theoretical at present. In some instances, epidemiological data can offer indirect evidence of a pathogen's transmissibility through blood by linking an increased incidence of a disease with a history of blood transfusion. For XMRV, there has been no data published linking CFS or prostate cancer to previous transfusions in patients.

Some of the controversy surrounding XMRV centres on the possibility that detection of the XMRV and/or MLV in human samples is due to contamination from mouse cells [40] or the commonly-used prostate cancer cell line CWR22Rv1. Many of the hundreds of mouse endogenous retroviruses present in the mouse genome may amplify with XMRV primers. As such, a few copies of the mouse genome may represent a substantial source of contamination.

If XMRV originated from a recombination of two proviruses in a prostate cancer cell line in the 1990s, as suggested by Paprotka et al. [10], this would invalidate the results published by Lombardi et al., as most of the patient samples in their study were obtained from a CFS outbreak in Nevada in the 1980s – i.e. before XMRV existed. All XMRV isolates reported to date are closely related to the viral sequence found in CWR22Rv1. Several researchers have noted that if the retrovirus had been replicating in humans, these sequences would contain much more variation.

XMRV shares extensive sequence identity with known xenotropic, nonecotropic and polytropic murine viruses; the first of these being known to infect many common human tumour cell lines. This is a phenomenon that has confused retrovirologists looking for disease associations for over three decades. Most putative associations of new or old human retroviruses with diseases (including CFS and prostate cancer) have turned out to be laboratory artefacts [41].

There are several previous examples of (retro)virus contamination. In 1975, virologist Robert Gallo reported a discovery of a new human virus called HL-23. This virus was eventually proven to be not one, but three different ape viruses (gibbon-ape virus, simian sarcoma virus and baboon endogenous virus). Gallo claims he has no idea how these viruses contaminated his research. In 1986, Max Essex announced the discovery of a new human AIDS virus called HTLV-4 which two years later turned out to be a monkey virus that had accidentally contaminated Essex's human blood samples. In the 1950s and 1960s, polio vaccines that were heavily contaminated with a cancer-causing monkey virus called SV-40 (simian virus 40) were given to millions of people. Over the decades, various studies have indicated that this virus is implicated in several forms of human cancer.

If the findings published by Lombardi et al. and Lo et al. are correct, the potential sources of discrepancies among studies currently published could arise from a number of factors, such as variations in study populations; geographic differences in prevalence; variance in case definition criteria and stages of illness, sample source and preparation, assay procedures, sensitivity and specificity of the test methods used; potential genetic variation of the virus and other unknown factors.

Conclusions

At present, there is not enough evidence to reliably assess the potential role of XMRV and MLVs in human pathology. Only two studies have been published so far reporting positive findings in CFS patients, in contrast to 13 studies in which no virus has been found. Many questions remain regarding the possible prevalence of XMRV in the human population, the incidence of XMRV in cases of CFS and the extent of genetic variation between XMRV isolates. It is also yet to be determined whether XMRV infection is a causal factor in the pathogenesis of a CFS subset, prostate cancer cases or any other disorder, or merely a passenger virus identified in immunocompromised patients and some normal subjects. It may also turn out to be simply the result of laboratory contamination.

Although it is theoretically presumed that XMRV could be transmitted through blood transfusion, no such transmission event has been identified, and there is no known evidence of XMRV/MLV infection, related illness or disease in transfusion recipients. XMRV may represent another emerging infectious agent that poses a risk to transfusion safety, and as with other agents, it is imperative that the action taken to ensure blood safety is swift and effective, yet based on the best available science. Currently, the scientific data are incomplete and conflicting. It seems likely that the findings in two studies on XMRV/MLV in CFS patients were due to laboratory contamination, and the majority of the evidence favours the conclusion that there is no causal relation between XMRV and CFS. With the development of validated assays, additional data will become available in the near future which will help inform decisions on blood donor eligibility and screening.

Considerations

ECDC would suggest the following for the Commission's consideration:

Donor screening

Implementation of blood donor screening at EU level is not recommended at this time as there is no published proof of transfusion transmission for these viruses and no consistent association with disease. Existing evidence strongly suggests that the original observations in some studies may have been due to laboratory artefacts. Validated assays that detect infected individuals but do not implicate non-infected individuals have not yet been developed.

Donor deferral

Deferrals of blood donors who have a history of CFS have been implemented in several countries as a precautionary measure until more data have been published. However, a critical examination of the existing evidence does not support implementing deferral to address known risk as there has been no study suggesting transfusion transmission. If implemented, the justification for the measure would be to apply an approach based purely on the precautionary principle. This measure could be re-examined after conclusions have been obtained from ongoing studies, possibly by the end of this year (2011).

At present, the evidence does not suggest any immediate risk of blood-borne transmission although it is suggested that the situation be continuously monitored until further information becomes available.

References

1. Devanur LD, Kerr JR. Chronic fatigue syndrome. J Clin Virol 2006;37(3):139-50.
2. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Kornaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med 1994;121(12):953-9.
3. Reeves WC, Wagner D, Nisenbaum R, Jones JF, Gurbaxani B, Solomon L et al., Chronic fatigue syndrome – a clinically empirical approach to its definition and study. BMC Med 2005;3:19.
4. Carruthers BM, Jain AK, De Meirleir KL, Peterson D, Klimas NG, Lerner AM et al. Myalgic encephalomyalitis/chronic fatigue syndrome: Clinical working case definition, diagnostic and treatment protocols. Journal of Chronic Fatigue Syndrome 2003;11(1):7-97.
5. Jason LA, Evans M, Porter N, Brown M, Brown A, Hunnell J et al. The Development of a Revised Canadian Myalgic Encephalomyelitis Chronic Fatigue Syndrome Case Definition. Am J Biochem Biotechnol 2010;6(2):120-135.
6. Holmes GP, Kaplan JE, Gantz NM, Komaroff AL, Schonberger LB, Straus SE, et al. Chronic fatigue syndrome: a working case definition. Ann Intern Med 1988;108(3):387-9.
7. Sharpe MC, Archard LC, Banatvala JE, Borysiewicz LK, Clare AW, David A et al. A report – chronic fatigue syndrome: guidelines for research. J R Soc Med 1991;84(2):118-21.
8. Urisman A, Molinaro RJ, Fischer N, Plummer SJ, Casey G, Klein EA et al. Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog 2006;2(3):e25.
9. Silverman RH, Nguyen C, Weight CJ, Klein EA. The human retrovirus XMRV in prostate cancer and chronic fatigue syndrome. Nat Rev Urol 2010;7(7):392-402.
10. Paprotka T, Delviks-Frankenberry KA, Cingöz O, Martinez A, Kung HJ, Tepper CG et al. Recombinant Origin of the Retrovirus XMRV. Science 2011 Jul 1 [cited 2011 Nov 16]. Available at: http://www.sciencemag.org/content/early/2011/05/31/science.1205292.full.pdf
11. Knouf EC, Metzger MJ, Mitchell PS, Arroyo JD, Chevillet JR, Tewari M et al. Multiple integrated copies and high-level production of the human retrovirus XMRV (xenotropic murine leukemia virus-related virus) from 22Rv1 prostate carcinoma cells. J Virol 2009;83(14):7353-6.
12. Stieler K, Schulz C, Lavanya M, Aepfelbacher M, Stocking C, Fischer N et al., Host range and cellular tropism of the human exogenous gammaretrovirus XMRV. Virology 2010;399(1):23-30.
13. Onlamoon N, Das Gupta J, Sharma P, Rogers K, Suppiah S, Rhea J et al. Infection, viral dissemination and antibody responses of Rhesus macaques exposed to the human gammaretrovirus XMRV. J Virol 2011 May [cited 2011 Nov 17]; 85(9):[4547-57]. Available at: http://jvi.asm.org/content/85/9/4547
14. Demettre E, Bastide L, D'Haese A, De Smet K, De Meirleir K, Tiev KP et al. Ribonuclease L proteolysis in peripheral blood mononuclear cells of chronic fatigue syndrome patients. J Biol Chem 2002;277(38):35746-51.
15. Suhadolnik RJ, Peterson DL, O'Brien K, Cheney PR, Herst CV, Reichenbach NL et al. Biochemical evidence for a novel low molecular weight 2-5A-dependent RNase L in chronic fatigue syndrome. J Interferon Cytokine Res 1997;17(7):377-85.
16. Lombardi VC, Ruscetti FW, Das Gupta J, Pfost MA, Hagen KS, Peterson DL et al. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science 2009;326(5952):585-9.
17. Erlwein O, Kaye S, McClure MO, Weber J, Wills G, Collier D et al. Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome. PLoS One 2010;5(1):e8519.
18. Groom HC, Boucherit VC, Makinson K, Randal E, Baptista S, Hagan S, et al. Absence of xenotropic murine leukaemia virus-related virus in UK patients with chronic fatigue syndrome. Retrovirology 2010;7:10.
19. van Kuppeveld FJ, de Jong AS, Lanke KH, Verhaegh GW, Melchers WJ, Swanink CM et al. 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 2010;340:c1018.
20. Switzer WM, Jia H, Hohn O, Zheng H, Tang S, Shankar A et al. 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 2010;7:57.
21. Lo SC, Pripuzova N, Li B, Komaroff AL, Hung GC, Wang R et al. Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. Proc Natl Acad Sci USA 2010;107(36):15874-9.
22. Hong P, Li J, Li Y. Failure to detect xenotropic murine leukaemia virus-related virus in Chinese patients with chronic fatigue syndrome. J Virol 2010;7:224.
23. Hohn O, Strohschein K, Brandt AU, Seeher S, Klein S, Kurth R, et al. No Evidence for XMRV in German CFS and MS Patients with Fatigue Despite the Ability of the Virus to Infect Human Blood Cells In Vitro. PLoS One 2010;[cited 2011 Nov 17];5(12). Available at: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0015632.
24. Henrich TJ, Li JZ, Felsenstein D, Kotton CN, Plenge RM, Pereyra F et al. Xenotropic murine leukemia virus-related virus prevalence in patients with chronic fatigue syndrome or chronic immunomodulatory conditions. J Infect Dis 2010;202(10):1478-81.

25. Satterfield BC, Garcia RA, Jia H, Tang S, Zheng H, Switzer WM et al. 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 2011;8(1):12.
26. Erlwein O, Robinson MJ, Kaye S, Wills G, Izui S, Wessely S et al. Investigation into the Presence of and Serological Response to XMRV in CFS Patients. PLoS One 2011 [cited 2011 Nov 17];6(3). Available at: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0017592
27. Furuta RA, Miyazawa T, Sugiyama T, Kuratsune H, Ikeda Y, Sato E et al. No association of xenotropic murine leukemia virus-related virus with prostate cancer or chronic fatigue syndrome in Japan. Retrovirology 2011;8:20.
28. Schutzer SE, Rounds MA, Natelson BH, Ecker DJ, Eshoo MW. Analysis of cerebrospinal fluid from chronic fatigue syndrome patients for multiple human ubiquitous viruses and xenotropic murine leukemia-related virus. Annals of Neurology 2011;69(4):735-738.
29. Shin CH, Bateman L, Schlaberg R, Bunker AM, Leonard CJ, Hughen RW et al. Absence of XMRV and other MLV-related viruses in patients with Chronic Fatigue Syndrome. J Virol 2011:JVI.00693-11.
30. Knox K, Carrigan D, Simmons G, Tegue F, Zhou Y Hackett J Jr et al. No Evidence of Murine-Like Gammaretroviruses in CFS Patients Previously Identified as XMRV-Infected. Science 2011;1;333(6038):94–7.
31. Robinson MJ, Erlwein OW, Kaye S, Weber J, Cingoz O, Patel A et al. Mouse DNA contamination in human tissue tested for XMRV. Retrovirology 2010;7:108.
32. Arnold RS, Makarova NV, Osunkoya AO, Suppiah S, Scott TA, Johnson NA et al. XMRV infection in patients with prostate cancer: novel serologic assay and correlation with PCR and FISH. Urology 2010;75(4):755-61.
33. Danielson BP, Ayala GE, Kimata JT. 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 2010;202(10):1470-7.
34. Fischer N, Hellwinkel O, Schulz C, Chun FK, Huland H, Aepfelbacher M et al. Prevalence of human gammaretrovirus XMRV in sporadic prostate cancer. J Clin Virol 2008;43(3):277-83.
35. Schlaberg R, Choe DJ, Brown KR, Thaker HM, Singh IR. XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. Proc Natl Acad Sci USA, 2009;106(38):16351-6.
36. Switzer WM, Jia H, Zheng H, Tang S, Heneine W. No Association of Xenotropic Murine Leukemia Virus-Related Viruses with Prostate Cancer. PLoS One 2011;[cited 2011 Nov 17]; 6(5). Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3087753/pdf/pone.0019065.pdf
37. Verhaegh GW, de Jong AS, Smit FP, Jannink SA, Melchers WJ, Schalken JA. Prevalence of human xenotropic murine leukemia virus-related gammaretrovirus (XMRV) in Dutch prostate cancer patients. Prostate 2011 Mar 1;71(4):415-20.
38. Sakuma T, Hué S, Squillace KA, Tonne JM, Blackburn PR, Ohmine S et al. No Evidence of XMRV in Prostate Cancer Cohorts in the Midwestern United States. Retrovirology 2011;8(1):23.
39. D'Arcy F, Foley R, Perry A, Marignol L, Lawler M, Gaffney E, et al. No evidence of XMRV in Irish prostate cancer patients with the R462Q mutation. European Urology Supplements 2008;7(3): 271-271.
40. Hohn O, Krause H, Barbarotto P, Niederstadt L, Beimforde N, Denner J et al. Lack of evidence for xenotropic murine leukemia virus-related virus(XMRV) in German prostate cancer patients. Retrovirology 2009;6: 92.
41. Martinez-Fierro ML, Leach RJ, Gomez-Guerra LS, Garza-Guajardo R, Johnson-Pais T, Beuten J et al. Identification of viral infections in the prostate and evaluation of their association with cancer. BMC Cancer 2010;10:326.
42. Garson J, Kellam P, Towers GJ. Analysis of XMRV integration sites from human prostate cancer tissues suggests PCR contamination rather than genuine human infection. Retrovirology 2011;8(1): 13.
43. Barnes E, Flanagan P, Brown A, Robinson N, Brown H, McClure M et al. Failure to detect xenotropic murine leukemia virus-related virus in blood of individuals at high risk of blood-borne viral infections. J Infect Dis 2010;202(10):1482-5.
44. Cornelissen M, Zorgdrager F, Blom P, Jurriaans S, Repping S, van Leeuwen E et al. Lack of detection of XMRV in seminal plasma from HIV-1 infected men in The Netherlands. PLoS One 2010;5(8): Available at: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0012040.
45. Gray ER, Garson JA, Breuer J, Edwards S, Kellam P, Pillay D et al. No Evidence of XMRV or Related Retroviruses in a London HIV-1-Positive Patient Cohort. PLoS One 2011;6(3): Available at: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0018096.
46. Kunstman KJ, Bhattacharya T, Flaherty J, Phair JP, Wolinsky SM. Absence of xenotropic murine leukemia virus-related virus in blood cells of men at risk for and infected with HIV. AIDS 2010;24(11): 1784-5.
47. Maggi F, Focosi D, Lanini L, Sbranti S, Mazzetti P, Macera L et al. Xenotropic murine leukaemia virus-related virus is not found in peripheral blood cells from treatment-naive human immunodeficiency virus-positive patients. Clinical Microbiology and Infection 2011 May 18. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1469-0691.2011.03580.x/full
48. Tang S, Zhao J, Viswanath R, Nyambi PN, Redd AD, Dastyar A et al. Absence of detectable xenotropic murine leukemia virus-related virus in plasma or peripheral blood mononuclear cells of human immunodeficiency virus Type 1-infected blood donors or individuals in Africa. Transfusion 2011;51(3): 463-8.
49. Maric R, Pedersen FS, Kjeldbjerg A, Moeller-Larsen A, Bahrami S, Brudek T et al. Absence of xenotropic murine leukaemia virus-related virus in Danish patients with multiple sclerosis. J Clin Virol 2010;49(3): 227-8.

50. Lintas C, Guidi F, Manzi B, Mancini A, Curatolo P, Persico AM. Lack of Infection with XMRV or Other MLV-Related Viruses in Blood, Post-Mortem Brains and Paternal Gametes of Autistic Individuals. PLoS One 2011 Feb 23;6(2):e16609.
51. Satterfield BC, Garcia RA, Gurrieri F, Schwartz CE. PCR and serology find no association between xenotropic murine leukemia virus-related virus (XMRV) and autism. Mol Autism 2010;1(1):14.
52. Jeziorski E, Foulongne V, Ludwig C, Louhaem D, Chiocchia G, Segondy M et al. No evidence for XMRV association in pediatric idiopathic diseases in France. Retrovirology 2010;7:63.
53. Luczkowiak J, Sierra O, González-Martín JJ, Herrero-Beaumont G, Delgado R. No Xenotropic Murine Leukemia Virus-related Virus Detected in Fibromyalgia Patients. Emerg Infect Dis 2011;17(2):314-5.
54. Martin, JJG, Herrero-Beaumont G, Delgado R. Lack of detection of Xenotropic murine leukemia virus-related virus (XMRV) in patients with fibromyalgia. Arthritis and Rheumatism, 2010:62;804.
55. McCormick AL, Brown RH Jr, Cudkowicz ME, Al-Chalabi A, Garson JA. Quantification of reverse transcriptase in ALS and elimination of a novel retroviral candidate. Neurology 2008;70(4):278-83.
56. Balada E, Castro-Marrero J, Felip L, Vilardell-Tarrés M, Ordi-Ros J. Xenotropic Murine Leukemia Virus-Related Virus (XMRV) in Patients with Systemic Lupus Erythematosus. Journal of Clinical Immunology 2011 Aug;31(4):584-7.
57. Fischer N, Schulz C, Stieler K, Hohn O, Lange C, Drosten C et al. Xenotropic murine leukemia virus-related gammaretrovirus in respiratory tract. Emerg Infect Dis 2010;16(6):1000–2.
58. Oakes B, Tai AK, Cingöz O, Henefield MH, Levine S, Coffin JM et al. Contamination of human DNA samples with mouse DNA can lead to false detection of XMRV-like sequences. Retrovirology 2010;7(1):109.
59. Hué S, Gray ER, Gall A, Katzourakis A, Tan CP, Houldcroft CJ et al. Disease-associated XMRV sequences are consistent with laboratory contamination. Retrovirology 2010;7(1):111.
60. Sato E, Furuta RA, Miyazawa T. An endogenous murine leukemia viral genome contaminant in a commercial RT-PCR Kit is amplified using standard primers for XMRV. Retrovirology 2010;7(1):110.
61. Tuke PW, Tettmar KI, Tamuri A, Stoye JP, Tedder RS et al. PCR Master Mixes Harbour Murine DNA Sequences. Caveat Emptor! PLoS One 2011;6(5): Available at: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0019953
62. Garson JA, Kellam P, Towers GJ. Analysis of XMRV integration sites from human prostate cancer tissues suggests PCR contamination rather than genuine human infection. Retrovirology 2011;8:13.
63. Qiu X, Swanson P, Luk KC, Tu B, Villinger F et al. Characterization of antibodies elicited by XMRV infection and development of immunoassays useful for epidemiologic studies. Retrovirology 2010;7:68.
64. Simmons G, Glynn SA, Holmberg JA, Coffin JM, Hewlett IK, Lo SC et al. The Blood Xenotropic Murine Leukemia Virus-Related Virus Scientific Research Working Group: mission, progress, and plans. Transfusion 2011;51(3):643–653.
65. Simmons G, Coffin J, Hewlett IK, Lo S-C, Mikovits J, Switzer WM et al. Multi-laboratory evaluations of XMRV nucleic acid detection assays. Retrovirology 2011;8(Suppl 1):A231.
66. XMRV: Testing the Blood Supply. Wall Street Journal 2011 Jan 20 [cited 2011-03-03]. Available at: http://blogs.wsj.com/health/2011/01/20/xmrv-testing-the-blood-supply/
67. Pathogenesis, Clinical and Public Health Implications. In: 1st International Workshop on XMRV [book of abstracts] 2010;7–8 September, Bethseda, USA.
68. Stoye JP, Silverman RH, Boucher CA, Le Grice SF. The Xenotropic Murine Leukemia Virus-Related Retrovirus Debate Continues at the First International Workshop. Retrovirology 2010;7(1):113.
69. Summary of the Oslo XMRV seminar, Nov 28, 2010 [cited 2011-03-03]. Available at: http://esme-eu.com/home/summary-of-the-oslo-xmrv-seminar-november-28th-2010-article467-6.html
70. Hanson M, Lee LL, Lin L, Bell DE, Ruppert D, Bell DS. Detection of MLV-like gag sequences in blood samples from a New York state CFS cohort. Retrovirology 2011;8(Suppl 1):A234.
71. Alberts B. Editorial Expression of Concern. Science, May 31 2011.
72. Chronic-Fatigue Paper Called Into Question. Wall Street Journal 2011 31 May;[cited 2011-05-31]. Available at: http://online.wsj.com/article/SB10001424052702303745304576355852212887170.html
73. Gallo vs Montagnier? Nature, 1991 Jun 6;351(6326):426.
74. The strains of the HIV war. Brown P. New Sci 1991 May 25;130(1770):20–1.
75. Culliton BJ. Deja vu in AIDS priority fight. Science 1990 Mar 30;247(4950):1532–3.
76. Cohen J. AIDS research. Longtime rivalry ends in collaboration. Science 2002;295(5559)1441–2.
77. Cohen J, Enserink M. Nobel Prize in Physiology or Medicine. HIV, HPV researchers honored, but one scientist is left out. Science 2008;322(5899):174–5.
78. Kanki PJ, Barin F, M'Boup S, Allan JS, Romet-Lemonne JL, Marlink R et al. New human T-lymphotropic retrovirus related to simian T-lymphotropic virus type III (STLV-IIIAGM). Science 1986;232(4747):238–43.
79. Hahn BH, Kong LI, Lee SW, Kumar P, Taylor ME, Arya SK et al. Relation of HTLV-4 to simian and human immunodeficiency-associated viruses. Nature 1987;330(6144):184-6.
80. Klein HG, Dodd RY, Hollinger FB, Katz LM, Kleinman S, McCleary KK et al. Xenotropic murine leukemia virus-related virus (XMRV) and blood transfusion: report of the AABB interorganizational XMRV task force. Transfusion, 2011 Mar;51(3):654–61.

81. American Red Cross Statement on XMRV and Chronic Fatigue Syndrome [cited 2011-03-03]. Available at: http://www.redcross.org/portal/site/en/menuitem.94aae335470e233f6cf911df43181aa0/?vgnextoid=dc099a02fbcac210VgnVCM10000089f0870aRCRD
82. Meeting of the FDA's Blood Products Advisory Committee (BPAC), December 14-15, 2010 [cited 2011-03-03]. Available at: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/BloodProductsAdvisoryCommittee/ucm239304.htm
83. Canadian Blood Services statement on Indefinite Deferral for History of Chronic Fatigue Syndrome [cited 2011-03-03]. Available at: http://www.bloodservices.ca/centreapps/internet/uw_v502_mainengine.nsf/9749ca80b75a038585256aa20060d703/db5c4e0235b819cc85257705006e5452?OpenDocument
84. Detailed Eligibility Criteria and FAQs. New Zealand Blood Services [cited 2011-03-03]. Available at: http://www.nzblood.co.nz/?t=65
85. The Australian Red Cross Blood Service statement on CFS donor policy [cited 2011-03-03]. Available at: http://www.donateblood.com.au/media-centre/latest-national-news/blood-service-updates-cfs-donor-policy
86. NHS Blood and Transplant Media Statement [cited 2011-03-03]. Available at: https://safe.blood.co.uk/PressRelease/MS033_081110_RG_ME_CFS_donor_deferral.pdf
87. Smith RA. Contamination of clinical specimens with MLV-encoding nucleic acids: implications for XMRV and other candidate human retroviruses. Retrovirology 2010;7:112.
88. Voisset C, Weiss RA and Griffiths DJ. Human RNA "rumor" viruses: the search for novel human retroviruses in chronic disease. Microbiol Mol Biol Rev 2008;72(1):157–96.

Annex 1. Search strategy

PUBMED

Concept 1	Boolean operator	Concept 2
OR		OR
'Xenotropic murine leukemia virus-related virus'[Mesh] 'xenotropic murine leukemia virus related virus'[Title/Abstract] 'xenotropic murine leukemia virus related virus infection'[Title/Abstract] 'xmrv'[Title/Abstract] 'xenotropic mulv related virus'[Title/Abstract] 'Xenotropic MuLV-related viruses'[Title/Abstract] 'xenotropic murine leukaemia virus related virus'[Title/Abstract] 'xenotropic murine leukemia related'[Title/Abstract] 'xenotropic murine leukemia retroviruses'[Title/Abstract] 'gammaretroviruses'[Title/Abstract] 'gammaretrovirus'[Title/Abstract] 'Gammaretrovirus'[Mesh] 'Retroviridae'[Mesh] 'retroviridae'[Title/Abstract] 'retrovirus'[Title/Abstract] 'retroviruses'[Title/Abstract]	AND	'Fatigue Syndrome, Chronic'[Mesh] 'akureyri'[Title/Abstract] 'akureyri disease'[Title/Abstract] 'royal free disease'[Title/Abstract] 'lake tahoe'[Title/Abstract] 'epstein barr virus reactivation'[Title/Abstract] 'chronic ebv'[Title/Abstract] 'chronic ebv infection'[Title/Abstract] 'chronic ebv infections'[Title/Abstract] 'iceland disease'[Title/Abstract] 'cfs'[Title/Abstract] 'mononucleosis like'[Title/Abstract] 'mononucleosis like disease'[Title/Abstract] 'mononucleosis like illness'[Title/Abstract] 'mononucleosis like illnesses'[Title/Abstract] 'mononucleosis like syndrome'[Title/Abstract] 'mononucleosis like syndromes'[Title/Abstract] 'chronic infectious mononucleosis syndrome'[Title/Abstract] 'chronic neuromyasthenia'[Title/Abstract] 'post viral fatigue'[Title/Abstract] 'post viral fatigue syndrome'[Title/Abstract] 'postviral fatigue syndromes'[Title/Abstract] 'epidemic neuromyasthenia'[Title/Abstract] 'neuromyasthenia'[Title/Abstract] 'neuromyasthenia chronic fatigue syndrome'[Title/Abstract] 'sporadic neuromyasthenia'[Title/Abstract] 'chronic asthenia'[Title/Abstract] 'chronic asthenia syndrome'[Title/Abstract] 'yuppie disease'[Title/Abstract] 'hyperfatigability'[Title/Abstract] 'chronic spasmophilia'[Title/Abstract] 'low nk cell'[Title/Abstract] 'myalgic encephalomyelitis'[Title/Abstract] 'myalgic encephalomyelitis syndrome'[Title/Abstract] 'myalgic encephalomyelitits'[Title/Abstract] 'myalgic encephalopathy'[Title/Abstract] 'chronic fatigue syndrome'[Title/Abstract] 'chronic fatigue syndrome/fibromyalgia'[Title/Abstract] 'chronic fatigue syndrome/fibromyalgia syndrome'[Title/Abstract] 'chronic fatigue syndrome/myalgic'[Title/Abstract] 'chronic fatigue syndrome/myalgic encephalitis'[Title/Abstract] 'chronic fatigue syndrome/myalgic encephalomyelitis'[Title/Abstract] 'chronic fatigue syndromes'[Title/Abstract] 'chronic fatigue and immune dysfunction syndrome'[Title/Abstract] 'chronic fatigue disorder'[Title/Abstract] 'chronic fatigue disorders'[Title/Abstract]

Limits: Publication date from 2006

EMBASE

Concept 1	Boolean operator	Concept 2
OR		OR
'xenotropic murine leukemia virus-related virus':ab 'xenotropic murine leukemia virus-related virus':ti 'xenotropic murine leukemia virus related virus infection':ab 'xenotropic murine leukemia virus related virus infection':ti xmrv:ab xmrv:ti 'xenotropic mulv related virus':ab 'xenotropic mulv related virus':ti 'xenotropic mulv-related viruses':ab 'xenotropic mulv-related viruses':ti 'xenotropic murine leukaemia virus related virus':ab 'xenotropic murine leukaemia virus related virus':ti 'xenotropic murine leukemia related':ab 'xenotropic murine leukemia related':ti 'xenotropic murine leukaemia related':ab 'xenotropic murine leukaemia related':ti 'xenotropic murine leukemia retroviruses':ab 'xenotropic murine leukemia retroviruses':ti 'xenotropic murine leukaemia retroviruses':ab 'xenotropic murine leukaemia retroviruses':ti 'gammaretrovirus infection'/exp 'leukemia virus'/exp gammaretrovirus:ab gammaretrovirus:ti gammaretroviruses:ab gammaretroviruses:ti 'leukemia virus':ab 'leukemia virus':ti 'murine leukemia virus'/exp 'retrovirus'/de 'murine leukemia virus':ab 'murine leukemia virus':ti	AND	'chronic fatigue syndrome'/exp 'chronic fatigue syndrome':ab 'chronic fatigue syndrome':ti akureyri:ab akureyri:ti 'royal free disease':ab 'royal free disease':ti 'lake tahoe':ab 'lake tahoe':ti 'epstein barr virus reactivation':ab 'epstein barr virus reactivation':ti 'chronic ebv':ab 'chronic ebv':ti 'iceland disease':ab 'iceland disease':ti cfs:ab cfs:ti 'mononucleosis like':ab 'mononucleosis like':ti 'chronic infectious mononucleosis syndrome':ab 'chronic infectious mononucleosis syndrome':ti 'chronic neuromyasthenia':ab 'chronic neuromyasthenia':ti 'post viral fatigue':ab 'post viral fatigue':ti 'epidemic neuromyasthenia':ab 'epidemic neuromyasthenia':ti 'neuromyasthenia chronic fatigue syndrome':ab 'neuromyasthenia chronic fatigue syndrome':ti 'sporadic neuromyasthenia':ab 'sporadic neuromyasthenia':ti 'chronic asthenia':ab 'chronic asthenia':ti 'yuppie disease':ab 'yuppie disease':ti 'hyperfatigability':ab 'hyperfatigability':ti 'chronic spasmophilia':ab 'chronic spasmophilia':ti 'low nk cell':ab 'low nk cell':ti 'myalgic encephalomyelitis':ab 'myalgic encephalomyelitis':ti 'myalgic encephalomyelitits':ab 'myalgic encephalomyelitits':ti 'myalgic encephalopathy':ab 'myalgic encephalopathy':ti 'chronic fatigue syndromes':ab 'chronic fatigue syndromes':ti 'chronic fatigue and immune dysfunction syndrome':ab 'chronic fatigue and immune dysfunction syndrome':ti 'chronic fatigue disorder':ab 'chronic fatigue disorder':ti

Limits: [embase]/lim AND [2006-2011]/py

Annex 2. Evidence tables

XMRV / MLV clinical studies in CFS patient populations (ordered by publication date)

Citation	Title	Study type	Methodology and cell type / fluid	Outcome and XMRV prevalence (positive results in **bold**)	Strengths and limitations
Lombardi VC et al. (2009). Science 326(5952): 585-589. [16]	Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome.	Clinical study, United States *Patients:* - 101 CFS patients (Fukuda [2] and Carruthers [4]) - 218 non-CFS controls.	*Methodology:* - Nested PCR (*gag*) - IFC with antibodies to MLV p30 (*gag*) - WB using SFFV antigens *Cell type/fluid:* - PBMCs - Serum.	*CFS patients:* - **68/101** (67%) by nested PCR (*gag*) - **7/11** (64%) by a second nested PCR (*gag* and *env*) - **19/30** (63%) MLV antigens - **9/18** (50%) antibodies to SFFV *env* proteins *Controls:* - **8/218** (3.7%) by nested PCR (*gag*) - **1/11** (9%) by a second nested PCR (*gag* but not *env*) - 0/16 (0%) MLV antigens - 0/7 (0%) antibodies to SFFV *env* proteins.	*Strengths:* - Several methods used *Limitations:* - Small population size - Questionable selection of patients [19] - No positive patient control samples.
Erlwein O et al. (2010). PLoS One 5(1): e8519. [17]	Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome.	Clinical study, United Kingdom *Patients:* - 186 CFS patients (Fukuda [2]).	*Methodology:* - Nested PCR for XMRV and MLV *Cell type/fluid:* - DNA extracted from whole blood.	*CFS patients:* - 0/186 (0%) by PCR for XMRV or MLV *Controls:* - No controls tested.	*Strengths:* - *Limitations:* - No patient control samples.
Groom HC et al. (2010). Retrovirology 7: 10. [18]	Absence of xenotropic murine leukaemia virus-related virus in UK patients with chronic fatigue syndrome.	Clinical study, United Kingdom *Patients:* - 170 CFS patients (Fukuda [2]) - 395 non-CFS controls (including 157 blood donors and patient samples) - Three different cohorts.	*Methodology:* - PCR (*gag* and *env*) - Real-time PCR (*env*), two primer sets - Serum viral neutralisation assay for XMRV/MLV *Cell type/fluid:* - PBMCs - Serum.	*CFS patients:* - 0/170 (0%) by PCR - **1/170** (0%) serology *Controls:* - 0/157 blood donors (0%) by PCR - **25/395** (6.3%) by serology.	*Strengths:* - *Limitations:* - Serology tests non-specific; majority of positives were in healthy controls - No positive patient control samples.
van Kuppeveld FJ et al. (2010). BMJ 340: c1018. [19]	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.	Clinical study, the Netherlands. *Patients:* - 32 CFS patients (Sharpe [7]) - 43 non-CFS controls.	*Methodology:* - Nested PCR (*gag*) - Real-time PCR (*int*) *Cell type/fluid:* - DMSO-frozen PBMCs.	*CFS patients:* - 0/32 (0%) *Controls:* - 0/43 (0%).	*Strengths:* - Matched case-control study *Limitations:* - Small population size - No positive patient control samples.
Switzer WM et al. (2010). Retrovirology 7: 57. [20]	Absence of evidence of xenotropic murine leukemia virus-related virus infection in persons with chronic fatigue syndrome and healthy controls in the United States.	Clinical study, United States. *Patients:* - 51 CFS patients (Reeves [3]) - 97 non-CFS controls (56 healthy controls, 41 blood donors).	*Methodology:* - Nested PCR (*gag* and *pol*) - WB (*gag* and *env* Ab) - EIA (*gag* and *env* Ab) - IFA *Cell type/fluid:* - PBMCs - Plasma.	*CFS patients:* - 0/51 (0%) *Controls:* - 0/97 (0%).	*Strengths:* - Tests performed in three labs *Limitations:* - Small population size - No positive patient control samples.

Citation	Title	Study type	Methodology and cell type / fluid	Outcome and XMRV prevalence (positive results in **bold**)	Strengths and limitations
Lo SC et al. (2010). Proc Natl Acad Sci USA 107(36): 15874-15879. [21]	Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors.	Clinical study, United States. *Patients:* - 37 CFS patients (Holmes [6] (25/37), Fukuda [2] (21 of the previous 25), unknown (12/37)) - 44 non-CFS controls (blood donors)	*Methodology:* - Nested PCR for XMRV/MLV (*gag*) - RT-PCR *Cell type/fluid:* - PBMCs - Plasma	*CFS patients:* - 0/37 (0%) for XMRV - **32/37** (86.5%) for MLV-related viruses *Controls:* - 0/44 (0%) for XMRV - **3/44** (6.8%) for MLV-related viruses	*Strengths:* - Using PCR primers from Lombardi study [16] *Limitations:* - Small population size - Negative controls from another geographical location - No positive patient control samples
Hong P et al. (2010). Virol J 7: 224. [22]	Failure to detect xenotropic murine leukaemia virus-related virus in Chinese patients with chronic fatigue syndrome.	Clinical study, China *Patients:* - 65 CFS patients (Fukuda [2]) - 85 non-CFS controls (65 blood donors, 20 with HBV, HCV, HIV and/or HTLV).	*Methodology:* - Multiplex PCR - RT-PCR *Cell type/fluid:* - PBMCs - Plasma.	*CFS patients:* - 0/65 (0%) *Controls:* - 0/85 (0%).	*Strengths:* - *Limitations:* - Small population size - No positive patient control samples.
Henrich TJ et al. (2010). J Infect Dis 202(10): 1478-1481. [24]	Xenotropic murine leukemia virus-related virus prevalence in patients with chronic fatigue syndrome or chronic immunomodulatory conditions	Clinical study, United States *Patients:* - 32 CFS patients (Fukuda [2]) - 261 non-CFS controls with various illnesses.	*Methodology:* - Nested PCR (*gag*) *Cell type/fluid:* - PBMCs.	*CFS patients:* - 0/32 (0%) *Controls:* - 0/261 (0%).	*Strengths:* - *Limitations:* - Small population size - No positive patient control samples.
Hohn O, Strohschein K et al. (2010). PLoS One 5(12): e15632. [23]	No Evidence for XMRV in German CFS and MS Patients with Fatigue Despite the Ability of the Virus to Infect Human Blood Cells In Vitro.	Clinical study, Germany *Patients:* - 39 CFS patients (Fukuda [2]) - 152 non-CFS controls (112 MS patients with fatigue, 40 healthy blood donors).	*Methodology:* - Nested PCR (*gag*) - EIA (*gag* and *env* Ab) *Cell type/fluid:* - PBMCs - Serum.	*CFS patients:* - 0/39 (0%) *Controls:* - 0/152 (0%).	*Strengths:* - *Limitations:* - Small population size - No positive patient control samples.
Satterfield BC, Garcia RA et al. (2011). Retrovirology 8(1): 12 [25]	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.	Clinical study, United States *Patients:* - 45 CFS patients (Fukuda [2]) - 42 non-CFS controls.	*Methodology:* - Real-time PCR (*pol*) - Nested PCR (*gag*) - WB *Cell type/fluid:* - PBMCs - Plasma.	*CFS patients:* - 0/45 (0%) *Controls:* - 0/42 (0%).	*Strengths:* - Blinded testing *Limitations:* - Small population size - No positive patient control samples.
Erlwein O, Robinson MJ et al. (2011). PLoS One 6(3): e17592 [26]	Investigation into the Presence of and Serological Response to XMRV in CFS Patients.	Clinical study, United Kingdom *Patients:* - 130 CFS patients from previous study [17] (Fukuda [2]) - 30 non-CFS controls.	*Methodology:* - Nested PCR (*gag*) - EIA to a related mouse retrovirus, New Zealand Black (NZB) gp70 *env* - Antigen capture assays based upon anti-MLV antibodies *Cell type/fluid:* - DNA from EDTA whole blood.	*CFS patients:* - 0/48 (0%) XMRV DNA sequences (*gag* and *env*) - 0/130 (0%) antibodies to MLV *env* protein - **4/130** (3%) elevated signals in an antigen capture assay for MLV (anti-Rauscher antibodies) - **20/130** (15%) elevated signals in an antigen capture assay for MLV (goat anti-NZB antibodies) *Controls:* - 0/30 (0%) antibodies to MLV *env* protein - **1/30** (3%) with elevated signals in an antigen capture assay for MLV. *Note: no serological reactions were considered to be specific.*	*Strengths:* - *Limitations:* - Small population size - No positive patient control samples.

Citation	Title	Study type	Methodology and cell type / fluid	Outcome and XMRV prevalence (positive results in **bold**)	Strengths and limitations
Furuta RA, Miyazawa T et al. (2011). Retrovirology 8: 20 [27]	No association of xenotropic murine leukemia virus-related virus with prostate cancer or chronic fatigue syndrome in Japan.	Clinical study, Japan *Patients:* - 100 CFS patients (Fukuda [2]) - 67 prostate cancer patients - 500 non-CFS controls (healthy blood donors).	*Methodology:* - RT-PCR *(gag)* - Nested PCR *(gag)* - Real-time PCR *(env)* - Immunoblot assay *Cell type/fluid:* - PBMCs - Plasma - Supernatants from co-cultured LNCap-FGC cells.	*CFS patients:* - 0/100 (0%) by PCR for XMRV gag - **2/100** (2%) antibodies to XMRV *gag* - 0/2 (0%) of antibody-positive samples positive by RT-PCR for XMRV *gag* - 0/2 (0%) of antibody-positive samples above positive by real-time PCR for XMRV *env* *Prostate cancer patients:* - **2/67** (3%) antibodies to XMRV *gag* *Controls:* - **8/500** (2%) antibodies to XMRV *gag*.	*Strengths:* - CFS patients were screened by genomic PCRs at three independent laboratories *Limitations:* - Patient controls not used for genetic analysis - No positive patient control samples.
Schutzer SE, Rounds MA et al. (2011). Annals of Neurology 69(4): 735-738. [28]	Analysis of cerebrospinal fluid from chronic fatigue syndrome patients for multiple human ubiquitous viruses and xenotropic murine leukemia-related virus.	Clinical study, United States *Patients:* - 43 CFS patients.	*Methodology:* - RT-PCR for XMRV (*gag* and *env*) *Cell type/fluid:* - CSF.	*CFS patients:* - 0/10 (0%) *gag* sequences - 0/43 (0%) *gag* or *env* sequences by RT-PCR or after co-cultivation with LNCaP cells in pools of 20 and 23 samples.	*Strengths:* - *Limitations:* - CSF only - CFS criteria unknown - Small population size - No patient control samples.
Shin CH, Bateman L et al. (2011). J. Virol.: JVI.00693-00611 [29]	Absence of XMRV and other MLV-related viruses in patients with chronic fatigue syndrome.	Clinical study, United States *Patients:* - 100 CFS patients (Fukuda [2] and Carruthers [4]) - 14 CFS patients from Lombardi study [16] (Fukuda [2]) - 200 non-CFS controls (healthy volunteers).	*Methodology:* - Real-time PCR for XMRV (*pol*, *gag* and *env*), four different assays - IAP assay for mouse DNA contamination - Single-round PCR for XMRV *(gag)* - Nested PCR for XMRV *(gag)* - EIA and WB for XMRV SU recombinant protein - Viral replication assay (culturing of patient plasma) *Cell type/fluid:* - PBMCs - Plasma - Serum.	*CFS patients:* - 0/100 (0%) by PCR, real-time PCR, EIA and WB - 0/31 (0%) in cultured cells by WB and real-time PCR *CFS patients from Lombardi study [16]:* - 0/14 (0%) by PCR, real-time PCR, EIA and WB. *Controls:* - 0/200 (0%) by PCR, real-time PCR, EIA and WB - 0/34 (0%) in cultured cells by WB and real-time PCR.	*Strengths:* - Addresses limitations from previous studies - CFS patient population fulfils both criteria from Lombardi study [16] - Wide range of assays used - Assays from Lombardi [16] and Lo [21] studies used - XMRV positive patients from Lombardi [16] study blindly tested, collected by third party - Very sensitive mouse DNA contamination test used - Controls from same geographical location *Limitations:* -
Knox K, Carrigan D et al. (2011). Science. [30]	No Evidence of Murine-Like Gammaretroviruses in CFS Patients Previously Identified as XMRV-Infected.	Clinical study, United States *Patients:* - 61 CFS patients in two groups; P1 (41) and P2 (29); nine patients were in both groups.	*Methodology:* - XMRV/MLV nested PCR (*gag* and *env*) - Nested RT-PCR (*gag*) - Viral culture and co-culture from PBMCs - Antibodies to XMRV p15E and gp70 by chemiluminescence. *Cell type/fluid:* - PBMCs - Plasma.	*CFS patients:* - 0/61 (0%) by PCR to any sequence - 0/29 (0%) by RT-PCR to any sequence - 0/29 (0%) infectious virus by culture - 0/60 (0%) antibody reactivity confirmed; one sample was weakly gp70 reactive but unconfirmed by WB.	*Strengths:* - Testing patients who have been previously tested positive for XMRV by WPI or VIPDx (Reno, NV) *Limitations:* - Small population size.