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Treatment for hepatitis C virus-associated mixed cryoglobulinaemia (Review)

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[Intervention Review]

Treatment for hepatitis C virus-associated mixed cryoglobulinaemia

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ABSTRACT

Background

Hepatitis C virus (HCV)-associated mixed cryoglobulinaemia is the manifestation of an inflammation of small and medium-sized vessels produced by a pathogenic IgM with rheumatoid factor activity generated by an expansion of B-cells. The immune complexes formed precipitate mainly in the skin, joints, kidneys or peripheral nerve fibres. Current therapeutic approaches are aimed at elimination of HCV infection, removal of cryoglobulins and also of the B-cell clonal expansions. The optimal treatment for it has not been established.

Objectives

This review aims to look at the benefits and harms of the currently available treatment options to treat the HCV-associated mixed cryoglobulinaemia with active manifestations of vasculitis (cutaneous or glomerulonephritis).

Search methods

We searched the Cochrane Kidney and Transplant Specialised Register to 30 November 2017 through contact with the Information Specialist using search terms relevant to this review. Studies in the Register are identified through searches of CENTRAL, MEDLINE, and EMBASE, conference proceedings, the International Clinical Trials Register (ICTRP) Search Portal and ClinicalTrials.gov.

Selection criteria

All randomised controlled trials (RCTs) and quasi-RCTs looking at interventions directed at treatment of HCV-associated cryoglobulinaemic vasculitis (immunosuppressive medications and plasma exchange therapy) have been included.

Data collection and analysis

Two authors independently assessed the retrieved titles and abstracts. Authors of included studies were contacted to obtain missing information. Statistical analyses were performed using random effects models and results expressed as risk ratio (RR) or mean difference (MD) with 95% confidence interval (CI). The planned primary outcomes were kidney disease, skin vasculitis, musculoskeletal symptoms, peripheral joint arthralgia, peripheral neuropathies, liver involvement, interstitial lung involvement, widespread vasculitis and death. Other planned outcomes were: therapy duration, laboratory findings, adverse effects, antiviral therapy failure, B-cell lymphoma, endocrine disorders and costs of treatment.

Main results

Ten studies were included in the review (394 participants). None of them evaluated direct-acting antivirals. Seven studies were single-centre studies and three were multicentre. The duration of the studies varied from six to 36 months. The risk of bias was generally unclear or low. Three different interventions were examined: use of rituximab (3 studies, 118 participants); interferon (IFN) (IFN compared to other

Treatment for hepatitis C virus-associated mixed cryoglobulinaemia (Review)

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strategies (5 studies, 223 participants); six IFN months versus one year (1 study, 36 participants), and immunoadsorption apheresis versus only immunosuppressive therapy (1 study, 17 participants).

The use of rituximab may slightly improve skin vasculitis (2 studies, 78 participants: RR 0.57, 95% CI 0.28 to 1.16; moderate certainty evidence) and made little or no difference to kidney disease (moderate certainty evidence). In terms of laboratory data, the effect of rituximab was uncertain for cryocrit (MD -2.01%, 95% CI -10.29% to 6.27%, low certainty evidence) and HCV replication. Rituximab may slightly increase infusion reactions compared to immunosuppressive medication (3 studies, 118 participants: RR 4.33, 95% CI 0.76 to 24.75, moderate certainty evidence) however discontinuations of the treatment due to adverse reactions were similar (3 studies, 118 participants: RR 0.97, 95% CI 0.22 to 4.36, moderate certainty evidence).

Effects of IFN on clinical symptoms were evaluated only in narrative results. When laboratory parameters were assessed, IFN made little or no difference in levels of alanine transaminase (ALT) at six months (2 studies, 39 participants: MD -5.89 U/L, 95% CI -55.77 to 43.99); rheumatoid factor activity at six months (1 study, 13 participants: MD 97.00 U/mL, 95% CI -187.37 to 381.37), or C4 levels at 18 months (2 studies, 49 participants: MD -0.04 mg/dL, 95% CI -2.74 to 2.67). On the other hand, at 18 months IFN may probably decrease ALT (2 studies, 39 participants: MD -28.28 U/L, 95% CI -48.03 to -8.54) and Ig M (-595.75 mg/dL, 95% CI -877.2 to -314.3), but all with low certainty evidence. One study reported infusion reactions may be higher in IFN group compared to immunosuppressive therapy (RR 27.82, 95% CI 1.72 to 449.18), and IFN may lead to higher discontinuations of the treatment due to adverse reactions (4 studies, 148 participants: RR 2.32, 95% CI 0.91 to 5.90) with low certainty evidence. Interferon therapy probably improved skin vasculitis (3 studies, 95 participants: RR 0.60, 95% CI 0.36 to 1.00) and proteinuria (2 studies, 49 participants: MD -1.98 g/24 h, 95% CI -2.89 to -1.07), without changing serum creatinine at 18 months (2 studies, 49 participants: MD -30.32 µmol/L, 95% CI -80.59 to 19.95).

Six months versus one year treatment with IFN resulted in differences terms of the maintenance of the response, 89% of patients in the six months group presented a relapse and only 11% maintained a long-term response at one year, while in the one year group only 78% relapsed and long-term response was observed in 22%. The one-year therapy was linked to a higher number of side-effects (severe enough to cause the discontinuation of treatment in two cases) than the six-month schedule.

One study reported immunoadsorption apheresis had uncertain effects on skin vasculitis (RR 0.44, 95% CI 0.05 to 4.02), peripheral neuropathies (RR 2.70, 95% CI 0.13 to 58.24), and peripheral joint arthralgia (RR 2.70, 95% CI 0.13 to 58.24), cryocrit (MD 0.01%, 95% CI -1.86 to 1.88) at six months, and no infusion reactions were reported. However when clinical scores were evaluated, they reported changes were more favourable in immunoadsorption apheresis with higher remission of severe clinical complications (80% versus 33%, $P = 0.05$) compared to immunosuppressive treatment alone.

In terms of death, it was not possible to present a pooled intervention effect estimate because most of the studies reported no deaths, or did not report death as an outcome.

Authors' conclusions

To treat HCV-associated mixed cryoglobulinaemia, it may be beneficial to eliminate HCV infection by using antiviral treatment and to stop the immune response by using rituximab. For skin vasculitis and for some laboratory findings, it may be appropriate to combine antiviral treatment with deletion of B-cell clonal expansions by using of rituximab. The applicability of evidence reviewed here is limited by the absence of any studies with direct-acting antivirals, which are urgently needed to guide therapy.

PLAIN LANGUAGE SUMMARY

Treatment for hepatitis C virus-associated cryoglobulinaemic vasculitis

What is the issue?

Nowadays, the second most frequent chronic viral infection in the world is hepatitis C virus (HCV) infection, before new direct-acting antiviral agents therapy has appeared. HCV causes chronic liver disease as well as extra-hepatic symptoms, one of the most common being mixed cryoglobulinaemia. Immunoglobulins that precipitate at low temperature (below 4°C) and re-dissolve after warming to 37°C are called cryoglobulins ("cryo" comes from the Greek word for cold). Cryoglobulins cause organ damage via two main routes: hyperviscosity syndrome and immune-mediated mechanisms. This causes cryoglobulinaemic vasculitis, which is the manifestation of an inflammation of small and medium-sized vessels produced by a pathogenic protein called IgM with rheumatoid factor activity generated by an expansion of B-cells. The immune complexes formed precipitate mainly in the skin, joints, kidneys and peripheral nerve fibres. We wanted to assess the effects of any treatment for skin, joints, kidneys or peripheral nerve fibres damage that occurs in HCV infection.

What did we do?

We identified 10 randomised controlled trials (RCT) enrolling 394 participants that met our inclusion criteria. This review looked at three possible strategies to control the disease and to investigate their impact on short and long-term outcomes: elimination of HCV infection by using interferon or new direct-acting antiviral agents, removal of cryoglobulins by using of immunoadsorption apheresis, or deletion of B-cell clonal expansions by using of rituximab. These strategies could be utilized in adults usually in combination with antiviral agents.

What did we find?

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Considering the limitation of the lack of studies with new direct-acting antiviral agents, some benefits in terms of skin, nerve or kidney illness were found when rituximab, antiviral treatment with IFN or immunoadsorption apheresis were used compared to using other immunosuppressive drugs. These three interventions were not compared head-to-head.

Conclusions

To treat HCV-associated mixed cryoglobulinaemia, it is always recommendable to eliminate HCV infection by using any antiviral treatment (in this review we only evaluated interferon treatment because no RCT with new antivirals looking at this disease have been published). Depending on the extent of mixed cryoglobulinaemia HCV-related disease it would be recommended to combine antiviral treatment with deletion of B-cell clonal expansions by using of rituximab or with removal of cryoglobulins by using of immunoadsorption because of their faster and more long-lasting improvement of skin, joints, kidneys or peripheral nerve fibres injury. Studies with new antivirals are urgently needed to guide therapy.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Rituximab compared to no rituximab for hepatitis C virus-associated mixed cryoglobulinaemia

Rituximab compared to no rituximab for hepatitis C virus-associated mixed cryoglobulinaemia

Patient or population: hepatitis C virus-associated mixed cryoglobulinaemia

Setting: USA and Italy

Intervention: rituximab

Comparison: no rituximab

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)
	Risk with no rituximab	Risk with rituximab			
Skin vasculitis (purpura, cutaneous ulcers or others) at 18 and 24 months: number of affected patients	Study population		RR 0.57 (0.28 to 1.16)	78 (2)	⊕⊕⊕⊕ MODERATE ¹
	26 per 100	15 per 100 (7 to 30)			
SCr at 18 months	The mean SCr at 18 months was 194.3 µmol/L	MD was 8.8 µmol/L lower (29.27 lower to 11.67 higher)	-	37 (1)	⊕⊕⊕⊕ MODERATE ¹
Cryocrit at 12 months assessed with: %	The mean cryocrit at 12 months was 6.55%	MD was 2.01% lower (10.29% lower to 6.27% higher)	-	41 (2)	⊕⊕⊕⊕ LOW ^{1 2}
Adverse effects - infusion reactions: number of events	Study population		RR 4.33 (0.76 to 24.75)	118 (3)	⊕⊕⊕⊕ MODERATE ¹
	0 per 100	0 per 100 (0 to 0)			
Activity outcomes BVAS: from 0 to 63	<p>De Vita 2012 found a significant reduction in the BVAS at 2 months in the rituximab group (from mean ± SD: 11.9 ± 5.4 to 7.1 ± 5.7; P < 0.001), and this difference persisted at 6 months (6.9 ± 6.8; P < 0.001), 12 months (5.4 ± 6.2; P 0.0001), and 24 months (4.4 ± 4.6; P < 0.0001). Without differences in the control group.</p> <p>Sneller 2012 BVAS scores became significantly lower in the rituximab group at month 4 (from 10.2 ± 8.4, at 6 months: 0 ± 0; P < 0.02). Without differences in control group.</p>		-	81 (2)	⊕⊕⊕⊕ MODERATE ³

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

BVAS: Birmingham vasculitis activity score; **CI:** confidence interval; **RR:** risk ratio; **SCr:** serum creatinine

GRADE Working Group grades of evidence

High quality: We are very confident that the true effect lies close to that of the estimate of the effect

Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

¹ 95% CI overlaps no effect, and the CI fails to exclude important benefit or important harm

² Base on tests of heterogeneity which test the null hypothesis that all studies have the same underlying magnitude of effect, have a low P-value ($P = 0.00001$), indicating to reject the null hypothesis I^2 statistic, which quantifies the proportion of the variation in point estimates due to among-study differences, is large (97%)

³ High risk of performance bias: non-blinded participants and personnel

BACKGROUND

Description of the condition

The second most frequent chronic viral infection in the world is hepatitis C virus (HCV) infection. Its estimated prevalence is 3% globally (around 170 million cases). HCV causes chronic liver disease as well as extra-hepatic symptoms such as lymphoproliferative disorders, kidney disease, or the most common, mixed cryoglobulinaemia. HCV is characterised by two remarkable immunologic characteristics: escape of immune response in more than 80% of infected people and production of monoclonal/polyclonal rheumatoid factor in 20% to 40% (Dammacco 2000).

Immunoglobulins that precipitate at low temperature (below 4°C) and re-dissolve after warming to 37°C are called cryoglobulins ("cryo" comes from the Greek word for cold). Cryoglobulins cause organ damage via two main routes: hyperviscosity syndrome and immune-mediated mechanisms. Although plasma cryoglobulins are detected in approximately 50% of HCV-infected patients, only 2% develop mixed cryoglobulinaemia systemic vasculitis (Charles 2009; Lauletta 2012; Saadoun 2008). This vasculitis is the manifestation of an inflammation of small and medium-sized vessels produced by a pathogenic IgM with rheumatoid factor activity generated by an expansion of B-cells (Dammacco 2010). The immune complexes formed precipitate mainly in the skin, joints, kidneys or peripheral nerve fibres. Disease manifestations are variable, ranging from moderate clinical symptoms (arthralgia, weakness that is nearly always present, lower limb palpable purpura, Raynaud's phenomenon, livedo reticularis, cutaneous ulcers, urticarial or oedema) or appearance of endocrine disorders (autoimmune thyroiditis, subclinical hypothyroidism, thyroid cancer or diabetes) to life threatening complications such as widespread vasculitis, glomerulonephritis (GN) or B-cell lymphoma (Ferri 2004; Matignon 2009; Saadoun 2008; Sansonno 2005; Sansonno 2007; Dammacco 2013).

Mixed cryoglobulinaemia is diagnosed by cryoglobulin testing, laboratory tests for evaluating visceral involvement and histopathological data to demonstrate a particular organ affection. The diagnosis requires demonstration of the presence of cryoglobulins in serum and typical organ involvement (Ramos-Casals 2012).

The demonstration of the role of HCV as the etiological factor in more than 90% of mixed cryoglobulinaemia patients has been critical to establish the treatment goals. Current therapeutic approaches are aimed at elimination of HCV infection, removal of cryoglobulins and also of the B-cell clonal expansions (Lauletta 2012).

Description of the intervention

The main possible interventions to treat mixed cryoglobulinaemia are eradicating the HCV infection and to attenuate immune response which leads to cryoglobulinaemic vasculitis by the use of different immunosuppressive medications, or extracorporeal therapies (including plasma exchange therapy, cryofiltration or immunoadsorption apheresis) used individually or in combination (Schwartz 2013; Siami 1995; Stefanutti 2009). However, the best strategy to treat this disease is not well defined.

Antiviral therapy against HCV with the combination of interferon (IFN)-alpha, pegylated (PEG)-IFN-alpha and ribavirin (a nucleoside antimetabolite agent) were the gold-standard for the treatment of HCV infection, but nowadays direct-acting antiviral agents (DAA) are becoming the mainstay to eradicating HCV infection (Desbois 2017; EASL 2011; Latt 2012; Martin 2017). The effectiveness of these strategies were demonstrated by a complete clinical response and sustained virological response (described as undetectable HCV RNA levels for at least 6 months after cessation of therapy) in 75% with DAA and 42.8% without DAA of HCV-related mixed cryoglobulinaemia patients (Cacoub 2017, Liang 2013; Lok 2012; Maasoumy 2013; Myers 2012).

There are also different possibilities of immunosuppressive treatments (corticosteroids or cyclophosphamide); biological therapies whose usefulness is only anecdotal such as tumour necrosis factor blockade by infliximab or etanercept (Bartolucci 2002; Chandesris 2004; Josselin 2008); anti-interleukin-6 therapy (Cohen 2012); IFN therapy (Kotter 2010); or rituximab (a chimeric monoclonal antibody specifically directed to CD20 antigen) (De Vita 2012; Sneller 2012). These strategies are sometimes ineffective, contraindicated, poorly tolerated, or may be associated with serious side effects (De Vita 2012). Therapeutic apheresis may be a useful option and can have immediate beneficial effects for the treatment of severe vasculitis or for clinical manifestations that are resistant to other treatments (Cacoub 2014; Pietrogrande 2011; Ramunni 2008; Schwartz 2013; Stefanutti 2009). There have been other approaches proposed for mixed cryoglobulinaemia, such as: imatinib (a tyrosine kinase inhibitor), thalidomide (an anti-angiogenic drug), bortezomib (a proteasome inhibitor) or proleukin (IL-2 therapy).

How the intervention might work

The principal target of any disease treatment is to remove the etiologic factor of the disorder. As a result of this assumption, in the case of HCV-related mixed cryoglobulinaemia, it seems that it is essential to eradicate the virus. However, the extrahepatic clinical manifestations of HCV infection can persist or relapse despite clearance of the HCV virus because they are mainly consequence of B-cell clonal expansions that can become independent of HCV stimulation (Langhans 2017; Schiavinato 2017). Consequently, B-cell clonal deletion with immunosuppression therapy may be a required step. The use of therapeutic apheresis is to remove a component of the blood which contributes to a disease state, in this case, cryoglobulins. Nevertheless, the concentration of cryoglobulin does not correlate with clinical severity or with response to therapy. Nowadays, the decision of immediate removal of cryoglobulins by plasma exchange is based on the severity of the disease manifestations, although it is unknown if it may have independent positive effects on survival.

Why it is important to do this review

Different therapeutic approaches in combination or alone have been assessed in several studies. Recent clinical research performed on the promising role of the biological agents or the therapeutic apheresis. Mixed cryoglobulinaemia involves important use of hospital resources, it entails morbidity and it also affects the quality of life of the HCV patients. The prognosis and natural history of mixed cryoglobulinaemia are variable. It principally depends on the extent of vasculitic lesions and kidney-associated disease. In fact, the overall five-year survival ranges

from 90% to 50% when kidneys are involved (Terrier 2013). Currently, there is no evidence-based strategy to manage mixed cryoglobulinaemia.

OBJECTIVES

This review aims to look at the benefits and harms of the currently available treatment options to treat the HCV-associated mixed cryoglobulinaemia with active manifestations of vasculitis (cutaneous or GN).

METHODS

Criteria for considering studies for this review

Types of studies

All randomised controlled trials (RCTs) and quasi-RCTs (RCTs in which allocation to treatment was obtained by alternation, use of alternate medical records, date of birth or other predictable methods) looking at interventions directed at treatment of HCV-associated mixed cryoglobulinaemia with active manifestations of vasculitis (immunosuppressive medications and plasma exchange therapy) have been included. There were no language restrictions.

Types of participants

Inclusion criteria

All patients with HCV-associated mixed cryoglobulinaemia (male or female), of any age, with or without chronic kidney disease (CKD) with active manifestations of vasculitis (cutaneous, peripheral neuropathy or GN).

The diagnosis of active vasculitis in patients with HCV-associated mixed cryoglobulinaemia could have been done following these criteria (Dammacco 2010): detection of serum cryoglobulins associated with purpura, arthralgia, and weakness; anti-HCV antibodies positivity and polymerase chain reaction-based assay to detect HCV RNA in serum; liver biopsy showing chronic hepatitis; negativity for hepatitis B surface antigen and human immunodeficiency virus. This review have not been limited by this criteria and accepted any definition described in the study reports.

Exclusion criteria

We excluded all studies focusing only on cryoglobulinaemic peripheral neuropathy.

Types of interventions

All possible comparisons between treatments have been eligible and have been analysed separately as well as in combination. These included the following.

1. Immunosuppressive medications (including rituximab, glucocorticoids, azathioprine, cyclophosphamide)
 - Different drug preparations
 - Different doses
 - Different frequency
 - Different duration
 - Different routes of administration
 - Monotherapy, dual therapy
2. Extracorporeal therapies
 - Plasma exchange therapy

- Cryofiltration or cryoglobulin apheresis
 - Immunoabsorption apheresis
3. Treatment for HCV (including IFN, PEG-IFN alpha-2b, ribavirin, boceprevir, telaprevir and other direct-acting antiviral agents).

Types of outcome measures

Primary outcomes

- Death
- Decreasing clinical manifestations (measured in different time points: 1, 6, 12 and 24 months)
 - Kidney disease (serum creatinine (SCr), glomerular filtration rate (GFR), proteinuria, active urinary sediment, need of dialysis, biopsy with GN)
 - Skin vasculitis (number and diameter of lesions, purpura, cutaneous ulcers or others)
 - Musculoskeletal symptoms (weakness (percentage of patients with complete response any time), peripheral joint arthralgia (percentage of patients with complete response any time) and peripheral neuropathies (percentage of patients with complete response any time))
 - Liver involvement (serum liver enzymes; echographic, histologic hepatitis or both; hepatocellular carcinoma)
 - Interstitial lung involvement characterized by subclinical alveolitis (presence of symptoms with radiologic, pulmonary function test alterations or both)
 - Widespread vasculitis involving medium-small sized arteries, capillaries and venules with multiple organ involvement: skin, kidney, lungs, central nervous system, and gastrointestinal tract (pain simulating an acute abdomen)

Secondary outcomes

- Duration of the therapy (months)
- Laboratory findings (at different time points): rheumatoid factor activity (IU/mL); quantification of C3, C4 and C1q levels (mg/dL); serum levels of IgA, IgG and IgM (mg/dL); type I, II and III cryoglobulin levels (mg/L); cryocrit percentage (%); B cell clonal expansion (CD20, CD19, CD20/CD5) in circulation (%); anti-HCV antibody titre (IU/L); serum HCV-RNA (IU/mL); fast glycaemia, cholesterol (total, LDL, HDL); thyroid function (thyrotropin, free thyroxine and triiodothyronine)
- Adverse effects of medication including: leukopenia (leukocytes/mL); thrombocytopenia (platelets/mL); aspartate aminotransferase (IU/L); total bilirubin (mg/dL); infusion reactions (number); discontinuations of treatment due to adverse drug reactions (number); infections (pneumonia, urosepsis); change in blood pressure; cardiovascular events (angina, myocardial infarction, heart failure); gastrointestinal bleeding; haemorrhagic alveolitis.
- Antiviral therapy failure or not indicated
- B-cell lymphoma
- Endocrine disorders (autoimmune thyroiditis, subclinical hypothyroidism, thyroid cancer or diabetes)
- Economic costs of treatment (including hospitalisation rate).

Search methods for identification of studies

Electronic searches

We searched the [Cochrane Kidney and Transplant Specialised Register](#) to 30 November 2017 through contact with the Information Specialist using search terms relevant to this review. The Cochrane Kidney and Transplant Specialised Register contains studies identified from several sources.

1. Monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL)
2. Weekly searches of MEDLINE OVID SP
3. Handsearching of kidney-related journals and the proceedings of major kidney conferences
4. Searching of the current year of EMBASE OVID SP
5. Weekly current awareness alerts for selected kidney and transplant journals
6. Searches of the International Clinical Trials Register (ICTRP) Search Portal and ClinicalTrials.gov.

Studies contained in the Specialised Register are identified through search strategies for CENTRAL, MEDLINE, and EMBASE based on the scope of Cochrane Kidney and Transplant. Details of these strategies, as well as a list of handsearched journals, conference proceedings and current awareness alerts, are available in the Specialised Register section of information about [Cochrane Kidney and Transplant](#).

See [Appendix 1](#) for search terms used in strategies for this review.

Searching other resources

1. Reference lists of clinical practice guidelines, review articles and relevant studies.
2. Email seeking information about unpublished or incomplete studies to investigators known to be involved in previous studies.

Data collection and analysis

Selection of studies

The search strategy described was used to obtain titles and abstracts of studies that may be relevant to the review. The titles and abstracts were screened independently by two authors, who discarded studies that were not applicable; however studies and reviews that might include relevant data or information on studies had been retained initially. Two authors independently assessed retrieved abstracts and, if necessary the full text, of these studies to determine which studies satisfied the inclusion criteria.

Data extraction and management

Data extraction was carried out independently by three authors using standard data extraction forms. It was planned to translate studies reported in non-English language journals before assessment, but at the end that was not necessary. Where more than one publication of one study existed, reports had been grouped together and the publication with the most complete data was used in the analyses. Where relevant outcomes were only published in earlier versions these data was used. Any discrepancy between published versions had been highlighted.

Assessment of risk of bias in included studies

The following items were independently assessed by two authors using the risk of bias assessment tool ([Higgins 2011](#)) (see [Appendix 2](#)).

- Was there adequate sequence generation (selection bias)?
- Was allocation adequately concealed (selection bias)?
- Was knowledge of the allocated interventions adequately prevented during the study?
 - Participants and personnel (performance bias)
 - Outcome assessors (detection bias)
- Were incomplete outcome data adequately addressed (attrition bias)?
- Are reports of the study free of suggestion of selective outcome reporting (reporting bias)?
- Was the study apparently free of other problems that could put it at a risk of bias?

Measures of treatment effect

For dichotomous outcomes (death, adverse effects of medications, active urinary sediment, need of dialysis, antiviral therapy failure) results have been expressed as risk ratio (RR) with 95% confidence intervals (CI). Where continuous scales of measurement were used to assess the effects of treatment (SCr, GFR, proteinuria, economic cost of the treatment, laboratory findings, duration of the therapy), the mean difference (MD) have been used, or the standardised mean difference (SMD) if different scales have been used. In case it was necessary to deal with change scores (decreasing global disease activity, reduction of the number or diameter of skin lesions), the statistical approach have been to include the baseline outcome measurements as a covariate in a regression model or analysis of covariance (ANCOVA) and have been included in the meta-analysis using the generic inverse-variance method. When the study outcomes were expressed using the median, the minimum and maximum values, in order to combine results, the sample mean and standard deviation (SD) was estimated using a published validated method ([Wan 2014](#)).

We approached time to event data expressing the intervention effect as a hazard ratio using to analyse it the generic inverse variance method. For counts and rates the results of a study was expressed as a RR and the (natural) logarithms of the rate ratios have been combined across studies using the generic inverse-variance method ([Higgins 2011](#)).

Unit of analysis issues

It was planned that cross-over studies would not be included, but we found one study and we analysed only the first part of the trial before the interventions were crossed. It was planned that, although it would have been highly unlikely to find other studies with non-standard designs, if a cluster-RCT had been included, statistical advice would have been asked for determining the most appropriate method to use and if studies with multiple intervention groups were included, they have been analysed combining groups to create a single pair-wise comparison.

Dealing with missing data

Any further information required from the original author was requested by written correspondence (e.g. emailing corresponding author) and any relevant information obtained in this manner was

included in the review. Evaluation of important numerical data such as screened, randomised patients as well as intention-to-treat, as-treated and per-protocol population have been carefully performed. Attrition rates, for example drop-outs, losses to follow-up and withdrawals have been investigated. Issues of missing data and imputation methods (for example, last-observation-carried-forward) have been critically appraised (Higgins 2011).

Assessment of heterogeneity

We first assessed the heterogeneity by visual inspection of the forest plot. Heterogeneity was then analysed using a Chi² test on N-1 degrees of freedom, with an alpha of 0.05 used for statistical significance and with the I² test (Higgins 2003). A guide to the interpretation of I² values is as follows.

- 0% to 40%: might not be important
- 30% to 60%: may represent moderate heterogeneity
- 50% to 90%: may represent substantial heterogeneity
- 75% to 100%: considerable heterogeneity.

The importance of the observed value of I² depends on the magnitude and direction of treatment effects and the strength of evidence for heterogeneity (e.g. P-value from the Chi² test, or a confidence interval for I²) (Higgins 2011).

Assessment of reporting biases

Funnel plots were to be used to assess for the potential existence of small study bias (Higgins 2011). There were insufficient studies to do this.

Data synthesis

Data have been pooled using the random-effects model but the fixed-effect model was also be used to ensure robustness of the model chosen and susceptibility to outliers.

Subgroup analysis and investigation of heterogeneity

Subgroup analyses have been used to explore possible sources of heterogeneity (participants, interventions and study quality). Heterogeneity among participants could be related to the HCV genotype (1 or 2) and efficacy of the antiviral agents therapy for HCV (IFN, ribavirin, boceprevir, telaprevir), age, liver involvement (considering all the possible criteria being used: severe versus non-severe liver fibrosis, i.e. F3-F4 versus F0-F2 in the Metavir scoring system or following a histologic criteria) or presence of chronic kidney disease. Heterogeneity in treatments could be related to prior immunosuppressive agent(s) used and the agent, previous administration of immunosuppressive drugs, concurrent use of extracorporeal therapies, the route of administration (e.g. whether oral or intravenous), the doses of drug given (if single or multiple doses) and the duration of the treatment (e.g. 1, 2, 6, 12 months). Adverse effects have been tabulated and assessed with descriptive techniques, as they were likely to be different for the various agents used. Where possible, the risk difference with 95% CI has been calculated for each adverse effect, either compared to no treatment or to another agent.

Sensitivity analysis

We planned to perform sensitivity analyses to explore the influence of the following factors on effect size.

- Repeating the analysis excluding unpublished studies
- Repeating the analysis taking account of risk of bias, as specified above
- Repeating the analysis excluding any very long or large studies to establish how much they dominate the results
- Repeating the analysis excluding studies using the following filters: diagnostic criteria, language of publication, source of funding (industry versus other), and country.

This was not possible due to the limited number of studies.

'Summary of findings' tables

We presented the main results of the review in 'Summary of findings' tables. These tables present key information concerning the quality of the evidence, the magnitude of the effects of the interventions examined, and the sum of the available data for the main outcomes (Schünemann 2011a). The 'Summary of findings' tables also include an overall grading of the evidence related to each of the main outcomes using the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) approach (GRADE 2008). The GRADE approach defines the quality of a body of evidence as the extent to which one can be confident that an estimate of effect or association is close to the true quantity of specific interest. The quality of a body of evidence involves consideration of within-trial risk of bias (methodological quality), directness of evidence, heterogeneity, precision of effect estimates and risk of publication bias (Schünemann 2011b). We presented the following outcomes in the Summary of findings table 1 evaluating the intervention comparing rituximab versus other immunosuppressive drugs.

- Clinical manifestations: skin vasculitis (purpura, cutaneous ulcers or others) at 18 and 24 months
- Laboratory findings: SCr (μmol/L) 18 months
- Laboratory findings: cryocrit (%) 12 months
- Adverse effects of the medication: infusion reactions (number)

RESULTS

Description of studies

Results of the search

After searching the Register we identified 33 records. No relevant titles were found by handsearching at clinicaltrials.gov (to date 30 November 2017) or in major conferences lists from 2000 to 2015 (American Society of Nephrology, European Renal Association-European Dialysis and Transplant Association, and International Society of Nephrology). After duplicates were removed and titles and abstracts screened we retrieved 23 full-text articles for further assessment. Of these, 10 studies (18 records) were included and 5 studies (5 records) were excluded (Figure 1).

Figure 1. Flow chart showing source and identification of studies for inclusion.

Included studies

See: [Characteristics of included studies](#)

Ten studies (394 participants) were included ([Dammacco 1994](#); [Dammacco 2010](#); [De Vita 2012](#); [Ferri 1991](#); [Mazzaro 1995](#); [Mazzaro 2000](#); [Misiani 1994](#); [Pioltelli 1995](#); [Sneller 2012](#); [Stefanutti 2009](#)). All studies were published in English.

One study author responded to queries about missing data stating he did not have the missing information ([Dammacco 1994](#)). No more responses were obtained.

Design

All the included studies were RCTS and were unblinded. The duration of the studies varied from six to 36 months. Seven studies were single-centre ([Dammacco 1994](#); [Dammacco 2010](#); [Ferri 1991](#); [Mazzaro 2000](#); [Misiani 1994](#); [Sneller 2012](#); [Stefanutti 2009](#)) and three were multicentre ([De Vita 2012](#); [Mazzaro 1995](#); [Pioltelli 1995](#)). One study was conducted in the USA ([Sneller 2012](#)), and the rest were conducted in Italy ([Dammacco 1994](#); [Dammacco 2010](#); [De Vita 2012](#); [Ferri 1991](#); [Mazzaro 1995](#); [Mazzaro 2000](#); [Misiani 1994](#); [Pioltelli 1995](#); [Stefanutti 2009](#)).

All the participants had the diagnosis of HCV-related mixed cryoglobulinaemia. In all studies potentially life-threatening vasculitis was an exclusion criterion. The median age of participants ranged between 50 to 70 years old and they were predominantly women.

Intervention and comparators

Three different interventions were found and analysed. Some studies included a combination of more than one agent.

Rituximab versus no rituximab treatment

Three studies (118 participants) compared rituximab with non-rituximab treatment ([Dammacco 2010](#); [De Vita 2012](#); [Sneller 2012](#)).

[Dammacco 2010](#) included antiviral treatment in both groups, while in the other two studies patient inclusion implied that therapy with antiviral agents had failed, had been poorly tolerated, or was considered to be contraindicated ([De Vita 2012](#); [Sneller 2012](#)).

[De Vita 2012](#) prescribed doses of rituximab was 1 g at day 1 and 15 (with standard premedication that included 100 mg of methylprednisolone) and only if relapse or clinical disease reappeared another course of medication was given only if there had been previous response to the drug. In the other two studies 375 mg/m² once a week for one month followed by two five-monthly infusions with premedication with 20 mg of 6-methylprednisolone in [Dammacco 2010](#), and without any steroids in [Sneller 2012](#).

Antiviral treatment was given simultaneously in both intervention groups using Peg-IFN-alpha (180 µg or 1.5 µg/kg) weekly plus ribavirin (1000 or 1200 mg) daily for 48 weeks ([Dammacco 2010](#)). The comparator groups in the two studies without antiviral treatment were with the previous immunosuppressive agents they had been receiving at the time of enrolment and were allowed to increase or initiate new ones as needed to manage worsening disease activity (glucocorticoids, azathioprine, cyclophosphamide or methotrexate) or plasma exchange.

Interferon treatment

Six studies (259 participants) evaluated the use of IFN. Five studies (223 participants) compared IFN with no IFN ([Dammacco 1994](#); [Ferri 1991](#); [Mazzaro 2000](#); [Misiani 1994](#); [Pioltelli 1995](#)), and one study (36 participants) compared the use PEG-IFN-alpha-2b for six months versus one year ([Mazzaro 1995](#)). Two studies compared the use of IFN versus maintaining previous immunosuppressive medications ([Ferri 1991](#); [Misiani 1994](#)). In [Mazzaro 2000](#), IFN was compared to oral prednisone at a dose of 0.2 mg/kg/d for six months, and [Pioltelli 1995](#) compared IFN-alpha-2b (3 MU, 3 times/week) versus deflazacort (1 or 0.2 mg/kg/d depending on the severity of disease) for at least two months and, if no improvement was obtained, was continued for a maximum of four months.

Other interventions

One study (17 participants) compared the use of immunoadsorption apheresis with immunosuppressive therapy versus only immunosuppressive therapy (Stefanutti 2009). The therapy also included PEG-IFN (3 MU, 3 times/week), cyclophosphamide (7.5 to 15 mg/kg, IV), CSA (2 to 5 mg/kg/d), ribavirin (10 to 15 mg/kg/d), or melphalan (0.1 to 0.2 mg/kg/d). The immunoadsorption apheresis was done using selesorb with a plasma volume exchange of 45 mL/Kg. The number of treatments for patients was between 24 and 35.

Outcomes

All studies were included in the meta-analyses.

None of the included studies reported: GRF; kidney biopsy (Mazzaro 2000 only reported basal kidney biopsy data); echographic or histologic hepatitis (Dammacco 1994 and Mazzaro 2000 reported basal data of liver biopsy); hepatocellular carcinoma; interstitial lung involvement; widespread vasculitis (Stefanutti 2009 reported basal data); C1q levels; IgG; type I, II and III cryoglobulin levels; CD20/CD5; fast glycaemia; cholesterol; thyroid function (thyrotropin, free thyroxine and triiodothyronine); total bilirubin; economic costs of treatment (including hospitalisation rate).

All studies reported death at different time-points.

Kidney involvement was reported differently in the studies: Dammacco 1994 referred to general improvement without specifying which was the improved parameter, and De Vita 2012 reported the presence of active urinary sediment. SCr, proteinuria and the need of dialysis was reported in the four studies (Dammacco 2010; De Vita 2012; Mazzaro 2000; Misiani 1994).

Six studies presented data on weakness, peripheral arthralgia, or peripheral neuropathies (Dammacco 1994; De Vita 2012; Ferri 1991; Mazzaro 1995; Misiani 1994; Stefanutti 2009), and eight studies reported data of skin vasculitis (Dammacco 1994; Dammacco 2010; De Vita 2012; Mazzaro 1995; Mazzaro 2000; Misiani 1994; Sneller 2012; Stefanutti 2009).

Some adverse events related to treatment were reported although this was not systematic. All reported withdrawal from study treatment, antiviral treatment failure and infusion reactions in rituximab group.

In De Vita 2012, the authors offered the patients in the non-rituximab group in whom treatment failed, the opportunity to be switched to rituximab in an open-label extension. At the end, 23/29 patients from the non-rituximab group switched to rituximab. We decided to perform intention-to-treat analyses, and the events of those switched patients are presented with the non-rituximab group.

Excluded studies

See: [Characteristics of excluded studies](#).

Five studies did not meet our inclusion criteria and were excluded because of the following reasons: one study was a protocol, with no results identified (Anonymous 1989), and four studies did not include participants meeting our inclusion criteria for HCV-associated mixed cryoglobulinaemia (Candela 1994; Ferri 1989; Lauti 1995; Vacca 1992).

Risk of bias in included studies

See [Characteristics of included studies](#) and [Figure 2](#) and [Figure 3](#). The risk of bias for many domains was generally unclear or low.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Dammacco 1994	?	?	-	?	+	+	+
Dammacco 2010	+	+	-	?	+	+	+
De Vita 2012	?	+	-	?	+	+	+
Ferri 1991	?	?	-	?	-	-	+
Mazzaro 1995	?	?	-	?	+	+	+
Mazzaro 2000	?	?	-	?	+	+	+
Misiani 1994	+	?	-	-	-	+	+
Pioltelli 1995	+	+	-	?	+	-	+
Sneller 2012	?	+	-	?	+	+	+
Stefanutti 2009	?	?	-	?	+	+	+

Figure 3. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

Allocation

Random sequence generation

Random sequence generation was judged to be at low risk of bias in three studies ([Dammacco 2010](#); [Misiani 1994](#); [Pioltelli 1995](#)) and unclear in seven studies ([Dammacco 1994](#); [De Vita 2012](#); [Ferri 1991](#); [Mazzaro 1995](#); [Mazzaro 2000](#); [Sneller 2012](#); [Stefanutti 2009](#)).

Allocation concealment

Allocation concealment was judged to be at low risk of bias in four studies ([Dammacco 2010](#); [De Vita 2012](#); [Pioltelli 1995](#); [Sneller 2012](#)) unclear in six studies ([Dammacco 1994](#); [Ferri 1991](#); [Mazzaro 1995](#); [Mazzaro 2000](#); [Misiani 1994](#); [Stefanutti 2009](#)).

Blinding

Performance bias (blinding of participants and investigators) was judged to be at high risk of bias for all 10 studies.

[Misiani 1994](#) that refers to "the examiner searched for signs of cutaneous vasculitis", which introduced a high risk of detection bias. The risk of bias was unclear in the remaining nine studies.

Incomplete outcome data

Two studies were judged to be at high risk of attrition bias ([Ferri 1991](#); [Misiani 1994](#)). [Ferri 1991](#) reported five patients had drop-out and the authors did not present data for them, and [Misiani 1994](#) reported that missing outcome data was balanced in number across intervention groups, but the authors did not impute these using appropriate methods. All other studies were judged to be at low risk of bias.

Selective reporting

Two studies were judged to be at high risk of reporting bias ([Ferri 1991](#); [Pioltelli 1995](#)). [Ferri 1991](#) specified that "renal and neurological involvement" would be evaluated however they did not report these outcomes, and in [Pioltelli 1995](#) there was no data at the end of the study of purpura, joint pain, weakness, kidney

impairment or neurological impairment. All other studies were judged to be at low risk of bias.

Other potential sources of bias

All studies appear to be free of other sources of bias.

Effects of interventions

See: [Summary of findings for the main comparison Rituximab compared to no rituximab for hepatitis C virus-associated mixed cryoglobulinaemia](#)

See: [Summary of findings for the main comparison](#) for the main comparison IFN versus other immunosuppressive drug for patients affected by mixed cryoglobulinaemia

There are some outcomes that it was planned to analyse as it was published in the protocol, but that has not been possible because they were not reported in the available studies.

Rituximab versus no rituximab treatment

Three studies compared rituximab with non-rituximab treatment ([Dammacco 2010](#); [De Vita 2012](#); [Sneller 2012](#)).

Death

Two studies reported 100% of survival ([Dammacco 2010](#); [Sneller 2012](#)). [De Vita 2012](#) found no differences in death between the groups at 24 months when intention-to-treat analysis was done: one in the non-rituximab group, two in the rituximab group, and three in the rituximab-switch group ([Analysis 1.1.3](#) (3 studies, 107 participants): RR 0.52, 95% CI 0.1 to 2.61; $I^2 = 0\%$).

Kidney disease

Two studies reported kidney disease parameters, however they did not report the same outcomes ([Dammacco 2010](#); [De Vita 2012](#)).

[De Vita 2012](#) (46 participants) reported little or no difference between rituximab and control for active urinary sediment at 1 month ([Analysis 1.2.1](#)) or the need for dialysis at 24 months

(Analysis 1.2.2). [Dammacco 2010](#) (37 participants) reported little or no difference between rituximab and control for SCr (Analysis 1.3.1; Analysis 1.3.2) and proteinuria (Analysis 1.3.1; Analysis 1.3.4) at 18 and 36 months. The mean proteinuria given in [Dammacco 2010](#) is a high value because authors only give results of those patients with a value of proteinuria > 0.25 g/24 h, and they were only three.

Skin vasculitis

Skin vasculitis was reported by two studies ([Dammacco 2010](#); [De Vita 2012](#)).

At one month [De Vita 2012](#) reported little or no difference between rituximab and control (Analysis 1.2.3). At 18 to 24 months rituximab may slightly improve skin vasculitis (Analysis 1.2.4 (2 studies, 78 participants): RR 0.57, 95% CI 0.28 to 1.16; $I^2 = 0\%$; moderate certainty evidence).

Rheumatoid factor activity

[De Vita 2012](#) described rheumatoid factor levels decreased at the end of six months of rituximab treatment (mean $\pm$ SD: 286.14 $\pm$ 67.81 IU/mL at baseline versus 122.31 $\pm$ 43.03 IU/mL at 6 months; $P = 0.0003$), with no differences between the randomised rituximab group and the rituximab-switch group (data is not presented in any analysis because the authors did not give any information of the control group).

Cryocrit

Two studies reported cryocrit (%) at 12 months ([Dammacco 2010](#); [Sneller 2012](#)). The mean and standard deviation was estimated from the median and interquartile range using ([Wan 2014](#)). The heterogeneity of I^2 of 97% when assessing the use of rituximab or not in terms of cryocrit variation (Analysis 1.3.5 (41 participants): MD -2.01%, 95% CI -10.29% to 6.27%; low certainty evidence) may indicate an error in the data because of inconsistent findings across studies.

HCV-RNA

[Sneller 2012](#) reported that rituximab treatment alone did not appear to affect HCV replication (numerical data is not given analysed because only mean values were reported). [Dammacco 2010](#) reported the use of antiviral treatment with or without rituximab might make no difference to viral replication (Analysis 1.3.9 (17 participants): MD -435182.00 IU/mL, 95% CI -1051224.79 to 180860.79).

Adverse effects

Three studies reported adverse effects ([Dammacco 2010](#); [De Vita 2012](#); [Sneller 2012](#)) however only infusion reactions and discontinuation of treatment could be meta-analysed.

Rituximab may slightly increase infusion reactions compared to other immunosuppressive medication (Analysis 1.4.1 (3 studies, 118 participants): RR 4.33, 95% CI 0.76 to 24.75; $I^2 = 0\%$; moderate certainty evidence), while there was little or no difference in discontinuation of treatment due to adverse reactions (Analysis 1.4.2 (3 studies, 118 participants): RR 0.97, 95% CI 0.22 to 4.36; $I^2 = 0\%$; moderate certainty evidence).

[Sneller 2012](#) reported no differences in infection (Analysis 1.4.3), leukopenia (Analysis 1.4.8) or thrombocytopenia (Analysis 1.4.9)

at six months. [De Vita 2012](#) reported no differences for infections (Analysis 1.4.4) (the authors found only four infections during the 24 months of the study: one in the rituximab group, one in the non-rituximab group, and two additional infection in rituximab switch group), cardiovascular events (Analysis 1.4.5) (one angina episode in rituximab group, one myocardial infarction and one heart failure episode in non-rituximab group, and three heart failure episodes in rituximab switch group), gastrointestinal bleeding (Analysis 1.4.6), or haemorrhagic alveolitis (Analysis 1.4.7) at 24 months.

Antiviral therapy failure

As it was previously described, antiviral failure or not indicated was one inclusion criteria in [De Vita 2012](#) and [Sneller 2012](#) studies, when we analysed data of this 2 studies, the use of rituximab makes little of no difference to antiviral failure (Analysis 1.5 (3 studies, 118 participants): RR 1.21, 95% CI 0.77 to 1.9), but what was interesting is that in [Dammacco 2010](#), the authors found that in rituximab group slightly increases the rate of antiviral failure compared to non-rituximab group (RR 2.85, 95% CI 1.44 to 5.62).

Other outcomes

The duration of therapy was always shorter in the rituximab group than in the control group and did not differ between studies.

Patients were treated for six months in control group and one month in rituximab group in [De Vita 2012](#). A second course of rituximab was administered to 15 patients who presented relapse. In [Sneller 2012](#), rituximab was given during 22 days and control group received 12 months of treatment. Both groups were receiving concomitant antiviral treatment for 12 months in [Dammacco 2010](#) and rituximab was given once a week for one month and then two, five-monthly infusions in the treatment group.

Activity outcomes

Although it was not initially planned we decided to include activity outcomes as they were the main comparison in the majority of included studies. The activity was quantified using the Birmingham Vasculitis Activity Score (BVAS), which is the most recognized validated instrument for standardised assessment of disease activity in vasculitis clinical trials.

[De Vita 2012](#) reported the global disease activity. They concluded that no difference in the BVAS was observed between the two treatment groups at baseline (mean $\pm$ SD: 9.6 $\pm$ 3.6 in the control group versus 11.9 $\pm$ 5.4 in the rituximab group; $P = 0.06$) or at two months (9.6 $\pm$ 4.5 in the control group versus 7.1 $\pm$ 5.7 in the rituximab group; $P = 0.076$). While no significant improvement in the non-rituximab group was observed for the BVAS at baseline versus two months ($P = 0.715$), a significant reduction in the BVAS at two months was seen in the rituximab group ($P < 0.001$), and this difference persisted at 6 months (6.9 $\pm$ 6.8; $P < 0.001$), 12 months (5.4 $\pm$ 6.2; $P < 0.0001$), and 24 months (4.4 $\pm$ 4.6; $P < 0.0001$).

[Sneller 2012](#) reported differences between groups. The authors explained that at baseline, BVAS were comparable between the two groups ($P = 0.977$), but became significantly lower in the rituximab group starting at four months with P values < 0.02.

Interferon versus control

Six studies evaluated the use of IFN ([Dammacco 1994](#); [Ferri 1991](#); [Mazzaro 1995](#); [Mazzaro 2000](#); [Misiani 1994](#); [Pioltelli 1995](#)).

To include the four groups from [Dammacco 1994](#), the approach used to overcome a unit-of-analysis error for a study that could contribute multiple, correlated, comparisons was to combine groups to create a single pair-wise comparison (IFN combined with IFN plus prednisone group (32 participants) and prednisone alone plus control group (33 participants)). Data from the cross-over trial of [Ferri 1991](#) was analysed by including only data from the first period because we considered that in this particular case, carry-over would be a problem. The numerical outcomes for [Misiani 1994](#) were presented using medians and minimum and maximum values, and in order to combine results, the mean and SD was estimated using a published validated method ([Wan 2014](#)).

Death

Four studies ([Dammacco 1994](#); [Ferri 1991](#); [Mazzaro 2000](#); [Pioltelli 1995](#)) reported no deaths at 12 and 24 months ([Analysis 2.1](#); [Analysis 2.2](#)).

Kidney disease

Two studies reported kidney disease parameters at 18 months ([Mazzaro 2000](#); [Misiani 1994](#)).

IFN made little or no difference to SCr at 18 months ([Analysis 2.3.1](#) (2 studies, 49 participants): MD -30.32 μ mol/L, 95% CI -80.59 to 19.95; $I^2 = 0\%$), however proteinuria was probably slightly reduced with IFN ([Analysis 2.3.2](#) (2 studies, 49 participants): MD -1.98 g/24 h, 95% CI -2.89 to -1.07; $I^2 = 0\%$).

Vasculitis

Five studies reported vasculitis ([Dammacco 1994](#); [Ferri 1991](#); [Mazzaro 2000](#); [Misiani 1994](#); [Pioltelli 1995](#)), however only three could be meta-analysed ([Dammacco 1994](#); [Ferri 1991](#); [Misiani 1994](#)).

IFN therapy probably reduced the skin vasculitis lesions compared to other immunosuppressive treatment ([Analysis 2.2](#) (3 studies, 95 participants): RR 0.60, 95% CI 0.36 to 1.00; $I^2 = 0\%$; moderate certainty evidence).

[Dammacco 1994](#) described that IFN controlled the activity in more than a half of patients and when it was combined with prednisone, a more prompt response and delayed relapse was found, but relapse was common after three months of treatment withdrawal from all treatment modalities. Similar results were found by [Mazzaro 2000](#), who described that both IFN (lymphoblastoid) and prednisone were associated with an improvement in clinical symptoms even in non-responders; complete recovery was achieved in only one patient in the IFN group, suggesting that complete remission could only be obtained through virus eradication. [Misiani 1994](#) concluded that the beneficial effect of IFN was limited to patients in whom HCV-RNA disappeared from the serum, whereas it worsened in those without response or in the control group. [Pioltelli 1995](#) did not find any difference between the effects of deflazacort versus IFN.

Liver involvement

IFN compared to control made little or no difference to ALT at six months ([Analysis 2.3.3](#) (2 studies, 39 participants): MD -5.89 UI/L, 95% CI -55.77 to 43.99; $I^2 = 93\%$), however IFN probably decreased ALT at 18 months ([Analysis 2.3.3](#) (2 studies, 39 participants): MD -28.28 UI/L, 95% CI -48.03 to -8.54; $I^2 = 0\%$).

Rheumatoid factor activity

[Mazzaro 2000](#) reported little or no difference between IFN and control in rheumatoid factor activity at 6 months ([Analysis 2.3.5](#) (13 participants): MD 97.00 UI/mL, 95% CI -187.37 to 381.37).

C4 levels

There was little no difference in C4 levels at 18 months ([Analysis 2.3.6](#) (2 studies, 49 participants): MD -0.04 mg/dL, 95% CI -2.74 to 2.67).

IgM

IFN probably decreased IgM at 18 months ([Analysis 2.3.7](#) (2 studies, 52 participants): MD -595.75 mg/dL, 95% CI -877.2 to -314.3; $I^2 = 0\%$).

Cryocrit

There was little or no difference in cryocrit at six months between IFN and control ([Analysis 2.3.8](#) (2 studies, 39 participants): MD -1.38%, 95% CI -2.38 to -0.38; $I^2 = 0\%$).

HCV-RNA

Three studies evaluated the effect of IFN on the serum HVC-RNA but their results could not be meta-analysed; [Misiani 1994](#) did not express the numerical data (only specified if it was positive or negative after the treatment) and the other two studies gave the results at different time points ([Mazzaro 2000](#) at 6 months and [Dammacco 1994](#) at 12 months).

Adverse effects

Five studies reported adverse effects ([Dammacco 1994](#); [Ferri 1991](#); [Mazzaro 2000](#); [Misiani 1994](#); [Pioltelli 1995](#)).

[Dammacco 1994](#) reported infusion reactions may be higher in IFN group compared to immunosuppressive therapy ([Analysis 2.4.1](#) (65 participants): RR 27.82, 95%CI 1.72 to 449.18, low certainty evidence).

IFN may lead to higher discontinuations of treatment due to adverse reactions ([Analysis 2.4.2](#) (4 studies, 148 participants): RR 2.32, 95% CI 0.91 to 5.90; $I^2 = 0\%$; low certainty evidence).

Antiviral failure

No difference was found in antiviral failure between groups ([Analysis 2.5](#) (2 studies, 78 participants) RR 0.59, 95% CI 0.21 to 1.64).

Other outcomes

In narrative results, [Mazzaro 2000](#) found minor side-effects only in the IFN group (fever, fatigue and a flu-like syndrome in most patients during the first two weeks) with one patient withdrawal (because of thrombocytopenia). [Misiani 1994](#) reported IFN was well tolerated except for influenza-like illness and two cases discontinued because of atrial fibrillation and depression. [Pioltelli 1995](#) described a higher incidence of major toxic effects in IFN group (25% of patients: one ictus cerebri, one refractory headache, one refractory fever, one refractory diarrhoea, one anaphylaxis episode, one impotentia coeundi, and one hypersomnia) versus 14% in deflazacort group (two femur fractures, one diabetes mellitus, one Cushing's syndrome).

The duration of the therapy was always similar between the two groups and it was not different between studies.

Interferon: six months versus one year treatment

One study compared six months of IFN with 12 months ([Mazzaro 1995](#)).

[Mazzaro 1995](#) reported no deaths at 24 months.

For the rest of available data, only narrative results can be presented.

- Complete response in 28% for six months duration treatment compared 39% of one year treatment; partial response was 50% in both groups and minor response was of 22% for six months and 11% for one year. Differences were observed in terms of the maintenance of the response, 89% of patients in the six months group presented a relapse and only 11% maintained a long-term response at one year, while in the one year group only 78% relapsed and long-term response was observed in 22%.
- The one-year therapy was linked to a higher number of side-effects (severe enough to cause the discontinuation of treatment in two cases) than the six-month schedule.

Immunoadsorption apheresis versus immunosuppressive drug therapy

One study compared immunoadsorption apheresis with immunosuppressive drug therapy ([Stefanutti 2009](#)).

[Stefanutti 2009](#) reported no deaths at 24 months. They also was also little or no difference between the treatments to skin vasculitis at six months ([Analysis 4.2.1](#)), peripheral neuropathies at six months ([Analysis 4.2.2](#)); peripheral joint arthralgia at six months ([Analysis 4.2.3](#)), cryocrit ([Analysis 4.3](#)), or to infusion reactions ([Analysis 4.4](#)).

[Stefanutti 2009](#) reported clinical score changes were more favourable in combined therapy with immunoadsorption apheresis plus immunosuppressants compared to immunosuppressive treatment alone (no numerical data). Higher remission of severe clinical complications was found with combined therapy (80% versus 33%, $P = 0.05$). It is also reported that "no adverse reactions or complications were noted".

DISCUSSION

Summary of main results

We have assessed a total of 10 studies involving 394 participants in this systematic review of treatment for hepatitis-C virus-associated mixed cryoglobulinaemia.

As discussed in other studies, the relevant clinical features to assess possible treatment strategies are kidney disease, skin vasculitis, musculoskeletal symptoms, and peripheral neuropathies. Moreover, the relevant biochemical parameters to assess immune response to the treatment are: C4 levels and the amount of cryoglobulins that can be estimated by quantifying the cryoglobulin levels, cryocrit, or rheumatoid factor. Three different interventions were examined: rituximab (three studies); IFN (six studies); and one study evaluated the use of immunoadsorption apheresis.

The majority of the studies under-reported relevant data or gave compound results that could not have been disaggregated, especially related to clinical outcomes. Although we send emails seeking information about that data to the study authors, we did not obtain any positive responses. As there were few events and participants for some outcomes, we found wide CIs crossing the line of no effect. For all the interventions, the effect on death was uncertain. Rituximab probably improved skin vasculitis wounds. All interventions make little or no difference to peripheral neuropathies, peripheral joint arthralgia, and kidney disease although IFN probably slightly reduced proteinuria as reported by [Mazzaro 2000](#) and [Misiani 1994](#). In narrative results, some authors reported that IFN improved clinical symptoms with a more prompt response and delayed relapse, however a study specified that the beneficial effect of IFN was limited to patients in whom HCV-RNA disappeared from the serum. Also in narrative description, clinical score changes were more favourable in combined therapy with immunoadsorption plus immunosuppressive therapy compared to immunosuppressive treatment alone, with higher remission of severe clinical complications found with combined therapy. Moreover, the use of rituximab improved BVAS scores, which became significantly lower at six months in the rituximab group. [Dammacco 2010](#) found that combining rituximab with antiviral treatment (IFN and ribavirin) significantly increased the rate of clinical response and sustained a higher long-term remission rate compared to antiviral treatment alone. There was uncertain direction of effect for cryocrit variation and for HCV replication when rituximab or immunoadsorption were assessed, but narrative results of [De Vita 2012](#) described that rheumatoid factor levels decreased at the end of month six after rituximab treatment. On the other hand, IFN may decrease ALT, IgM at 18 months and cryocrit at six months. Rituximab or immunoadsorption plus other immunosuppressive strategies made no difference to infusion reactions or discontinuations of the treatment due to adverse reactions. However, infusion reactions, discontinuation of the treatment and side effects were probably higher in IFN group compared to immunosuppressive therapy.

It seems logical that to treat HCV-associated mixed cryoglobulinaemia, one of the main aspects might be to remove its disease causative agent with antiviral therapy. Our results show that it may be beneficial to eliminate HCV infection by using antiviral treatment to improve the vasculitis signs and symptoms. However, in this review we only have been able to evaluate IFN treatment because no RCT with new antivirals looking at this disease have been published, although cohort studies and case reports demonstrated an achievement of a sustained virological response and complete clinical and immunological remission ([Cornella 2015](#); [Gragnani 2016](#); [Saadoun 2016](#); [Sise 2016](#); [Suwanhawornkul 2015](#)). See characteristics of these cohort studies in [Table 1](#). For skin vasculitis and for some laboratory findings, it may be appropriate to combine antiviral treatment with deletion of B-cell clonal expansions by using of rituximab.

Overall completeness and applicability of evidence

The main problem of this review is that the treatment of HCV-associated mixed cryoglobulinaemia has changed during the last months. Treatment with new direct-acting antivirals has shown high cure rates ([Cacoub 2017](#)) but the absence of published or ongoing trials of these new antivirals in this population did not let us include them in our systematic review, although we know that

this therapy is nowadays the mainstay of the treatment. Another problem has been that the data was incomplete in several areas despite our comprehensive search and direct contact with all of the authors of published studies; data found sometimes could not have been meta-analysed.

Quality of the evidence

Due to the lack of studies on the topic that combined antiviral treatment with other interventions or RCTs that evaluate new antivirals that during last year has been demonstrated to be the gold-standard to treat HCV infection, our conclusions must be considered cautiously because of several potential limitations in the available data.

We are aware that the width of the CIs could reflect a lack of statistical power which is directly related to the sample size of the included studies that evaluated the same outcomes that could be meta-analysed.

Potential biases in the review process

This review has three main limitations. Firstly, there are currently no RCTs using new antiviral agents on this topic. Secondly, no study included patients in the worse scenario of mixed cryoglobulinaemia (severe renal disease or potential life threatening complications) which imply there is no evidence about the best treatment strategy for these cases. Lastly, the evaluation of other interventions (rituximab, deflazacort or immunoadsorption apheresis) was without being combined with antiviral treatment except in [Dammacco 2010](#). Two out of the three included studies who assessed rituximab ([De Vita 2012](#); [Sneller 2012](#)) enrolled patients in whom antiviral therapy had failed to induce remission,

either because of a lack of sustained virologic response or regimen-related toxicity. Limiting our study to only one study with combined therapy made it not possible to assess the efficacy and effect of rituximab on the underlying HCV infection with concomitant antiviral therapy.

Agreements and disagreements with other studies or reviews

No previous systematic reviews have been published on this topic but during the last two years, some narrative reviews assessed this subject ([Cacoub 2014](#); [Cacoub 2015](#); [Dammacco 2016](#); [Terrier 2013](#)) and their results did not differ from the ones presented in this review.

AUTHORS' CONCLUSIONS

Implications for practice

There is currently insufficient evidence to determine the best strategy to treat HCV-associated mixed cryoglobulinaemia apart from antiviral treatment.

Implications for research

To assess the immunomodulation using the new antiviral treatment is now an important question to resolve.

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CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by study ID]

Dammacco 1994

Methods	<ul style="list-style-type: none"> Study design: parallel RCT Time frame: 1989 to 1992 Follow-up period: 29 months
Participants	<ul style="list-style-type: none"> Country: Italy Setting: single centre Inclusion criteria: patients with detectable serum cryoglobulins for at least 1 year, associated with purpura, weakness and arthralgia Number: treatment group 1 (15); treatment group 2 (18); treatment group 3 (17); control group (15) Mean age $\pm$ SD (years): treatment group 1 (59.2 $\pm$ 7.08); treatment group 2 (49.4 $\pm$ 16.9); treatment group 3 (56.3 $\pm$ 3.3); control group (51.4 $\pm$ 5) Sex (F/M): treatment group 1 (9/6); treatment group 2 (10/8); treatment group 3 (9/8); control group (8/7)

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Dammacco 1994 (Continued)

	<ul style="list-style-type: none"> Exclusion criteria: IFN or immunosuppressive therapies within the previous 6 months; pregnancy; concomitant serious illness that might preclude completion of the study; hepatic failure characterized by a history of ascites, bleeding oesophageal varices, endogenous hepatic encephalopathy, serum bilirubin level > 3 mg/dL, serum albumin level < 3 g/dL, and prothrombin time greater than 3 sec longer than that of the control; leukocyte count < 3 x 10⁹/L and/or a platelet count < 70 x 10⁹/L or Hb < 10 g/dL; positive test for hepatitis B surface antigen and/or antibodies to HIV; Epstein-Barr virus or CMV acute or chronic infection; connective tissue disorders; Waldenström's macroglobulinaemia or lymphoma
Interventions	<p>Treatment group 1</p> <ul style="list-style-type: none"> IFN alpha: 3 MU (IM) 3 times/week for 48 weeks <p>Treatment group 2</p> <ul style="list-style-type: none"> Prednisone: 16 mg/d for 48 weeks <p>Treatment group 3</p> <ul style="list-style-type: none"> IFN alpha: 3 MU (IM) 3 times/week for 48 weeks Prednisone: 16 mg/d on non-IFN days <p>Control group</p> <ul style="list-style-type: none"> No treatment
Outcomes	<ul style="list-style-type: none"> Response to the treatment <ul style="list-style-type: none"> Complete response: reduction of cryocrit < 50% initial value associated with one or more of the following: disappearance of purpura, improvement of neuropathy or improvement of kidney involvement Partial response: 50% to 10% reduction of cryocrit and one or more but not all clinical and laboratory signs of mixed cryoglobulinaemia. Relapse: increase of > 50% cryocrit after treatment HCV RNA titres Drug toxicity
Notes	<ul style="list-style-type: none"> In IFN controls the activity in more than a half of patients and when it is combined with prednisone, a more prompt response and delayed relapse is found Prednisone induced an increase of viraemia Relapse was common after 3 months of treatment withdrawal from all treatment modalities This study with multiple intervention groups has been analysed combining groups to create a single pair-wise comparison (with IFN: Group 1 (IFN) + Group 3 (IFN+prednisone) and without: Group 2 (prednisone) + Group 4 (control))

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "Randomly assigned"; method of randomisation not reported
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgement
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label study

Dammacco 1994 (Continued)

Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient information to permit judgement
Incomplete outcome data (attrition bias) All outcomes	Low risk	No missing data
Selective reporting (reporting bias)	Low risk	Results are clearly stated
Other bias	Low risk	Study appears free of other biases

Dammacco 2010

Methods	<ul style="list-style-type: none"> Study design: parallel RCT Time frame: January 2003 to June 2009 Follow-up period: 36 months
Participants	<ul style="list-style-type: none"> Country: Italy Setting: single centre Inclusion criteria: detection of serum cryoglobulins associated with the triad purpura, arthralgia, and weakness; positivity for anti-HCV antibodies and PCR-based assay to detect HCV RNA in serum; liver biopsy showing chronic hepatitis (performed within 3 months from enrolment); negativity for hepatitis B surface antigen and HIV and no previous administration of IFN or immunosuppressive drugs Number: treatment group 1 (22); treatment group 2 (15) Median age, range (years): treatment group 1 (63, 51 to 68); treatment group 2 (59, 50 to 66) Sex (F/M): treatment group 1 (15/7); treatment group 2 (10/5) Exclusion criteria: histologically proven biliary, neoplastic, and vascular liver diseases; psychiatric disorders, a history of seizures, cardiovascular diseases, poorly controlled diabetes mellitus; frank autoimmune disorders and metabolic liver disease; ingestion of more than 40 g alcohol/d; use of hepatotoxic drugs in the last 6 months; pregnancy; refuse to provide informed consent
Interventions	<p>Treatment group 1</p> <ul style="list-style-type: none"> Peg-IFN-alpha: 180 µg or 1.5 µg/kg weekly for 48 weeks Ribavirin: 1000 or 1200 mg daily for 48 weeks Rituximab: 375 mg/m² once a week for 1 month followed by two 5-monthly infusions. Rituximab pre-medication included 20 mg of 6-methyl-prednisolone. <p>Treatment group 2</p> <ul style="list-style-type: none"> Peg-IFN-alpha: 180 µg or 1.5 µg/kg weekly for 48 weeks Ribavirin: 1000 or 1200 mg daily for 48 weeks
Outcomes	<ul style="list-style-type: none"> Proportion of complete response at any time based on disappearance or remarkable improvement of clinical features, disappearance of cryoglobulins (immunologic response), undetectability of serum HCV-RNA (virologic response), and disappearance of B-cell clonalities from the blood (molecular response) Partial response (arbitrarily defined as the achievement of 3 of the 4 mentioned complete response criteria) Proportion of sustained complete response: stable at month 6 or until month 36 after completion of therapy

Dammacco 2010 (Continued)

- Notes
- After 1 year, complete response was achieved in 54.5% in group 1 and 33.3% in group 2 ($P < 0.05$) and it occurred within the 10 month for both treatments
 - Of these patients with complete response, relapse occurred in 16.6% and in 60% (3/5) of group 1 and group 2, respectively

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation assignment was computer-generated by an off-site biostatistician using block sizes of 2
Allocation concealment (selection bias)	Low risk	Patient assignments were sealed in opaque envelopes that were marked on the outside with a sequence number
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label study
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient information to permit judgement
Incomplete outcome data (attrition bias) All outcomes	Low risk	Quote: "All 37 patients completed the study".
Selective reporting (reporting bias)	Low risk	Outcomes well reported.
Other bias	Low risk	Study appears free of other biases

De Vita 2012

- Methods
- Study design: parallel RCT
 - Time frame: not reported
 - Follow-up period: 24 months
- Participants
- Country: Italy
 - Setting: multicentre (number of centres not listed)
 - Inclusion criteria: patients with severe mixed cryoglobulinaemia who had type II cryoglobulins either HCV-related or -unrelated (there were 4 patients with HCV unrelated mixed cryoglobulinaemia 1 in rituximab group and 3 in the control group), classified according to published criteria, and had serum cryoglobulins. In patients with HCV-related cryoglobulinaemic vasculitis, study inclusion implied that therapy with antiviral agents with IFN plus ribavirin had failed, had been poorly tolerated, or was considered to be contraindicated
 - Number: treatment group (28); control group (29)
 - Mean age $\pm$ SD (years): treatment group (62.85 ± 11.36); control group (63 ± 10.6)
 - Sex (F/M): treatment group (24/4); control group (22/7),
 - HCV positive: 53/57 (93%), they were no differences between the groups in terms of disease activity
 - Exclusion criteria: presence of antibodies against human immunodeficiency virus and hepatitis B virus core antigen and surface antigen.

De Vita 2012 (Continued)

Interventions	<p>Treatment group</p> <ul style="list-style-type: none"> Rituximab: 1 g IV at day 0 and 14 with standard premedication (100 mg IV methylprednisolone, 1000 mg oral acetaminophen, 10 mg IV chlorpheniramine maleate administered before each infusion). At the 6th month after initiation of rituximab, if no response was observed, rituximab was withdrawn <p>Control groups (non-rituximab)</p> <ul style="list-style-type: none"> High-doses of corticosteroids in 17/29 (58.6%): a maximum initial dosage of 1 mg/kg/d of prednisone or equivalent, with or without preceding 6 pulse doses of methylprednisolone (500 to 1000 mg/d for 3 consecutive days), and with subsequent reduction of the glucocorticoid dosage during the following months Cyclophosphamide in 4/29 (13.8%) and azathioprine in 3/29 (10.3%): given orally at a dosage of 1 to 2 mg/kg/d, with or without glucocorticoids (as above) Plasmapheresis in 5/29 (17.2%): at least 2 procedures/week during the first month, with subsequent reductions according to local protocols, with or without glucocorticoids (as above). <p>Both groups</p> <ul style="list-style-type: none"> If a response was observed, any of the treatments might be suspended after the end of month 6 following randomisation and then reintroduced if clinical relapse occurred
Outcomes	<ul style="list-style-type: none"> Proportion of patients who continued to take the treatment to which they were randomised at the end of month 12 (after randomisation) Survival of treatment at the end of 24 month after randomizations Decreasing of global disease activity (defined by BVAS) Assessment of side effects Response rate in rituximab-switched group
Notes	<ul style="list-style-type: none"> Enrolment was stopped when the sample was only of 42 patients, although the sample size calculated was of 124 patients. That was because of the superiority of rituximab versus non-rituximab for the primary end-point (63.2% versus 4.4%, (95% CI 35.6 to 82.0); P 0.0001) during the first 12-month interim analysis. Reasons for treatment failure up to month 24 in patients in the non-rituximab group were: a lack of response or worsening of target organ manifestations in 86.2% of patients and side effects in 10.3%. Reasons for treatment failure during the same period in patients in the rituximab group were a lack of response or worsening of target organ manifestations in 14.3% of patients and side effects in 14.3%. Three of the 28 patients in the rituximab group (all of whom had responded to the therapy), were lost to follow-up from month 12 of the study

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "randomised at a ratio of 1:1"; method of randomisation not reported
Allocation concealment (selection bias)	Low risk	Insufficient information to permit judgement
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label study
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient information to permit judgement

De Vita 2012 (Continued)

Incomplete outcome data (attrition bias) All outcomes	Low risk	Intention-to-treat analysis. 59 patients randomised. From non-rituximab group, 23 patients were rescued with rituximab and from rituximab group, 3 patients lost to follow-up but all data is presented
Selective reporting (reporting bias)	Low risk	All data referred in the "methods section" is presented
Other bias	Low risk	Unfunded study. Roche provided the study medication but, "Roche had no role in the study design or in the collection, analysis, or interpretation of the data"

Ferri 1991

Methods	<ul style="list-style-type: none"> Study design: cross-over RCT Time frame: not reported Follow-up period: 13 months
Participants	<ul style="list-style-type: none"> Country: Italy Setting: single centre Inclusion criteria: patients with at least 2 of the typical symptoms (purpura, arthralgia, or weakness), a measurable cryocrit level and liver and/or neurologic involvement Number: 26 Median age $\pm$ SD: 54 $\pm$ 6 years Sex (F/M): 15/11 Mean duration of disease: 11.2 $\pm$ 5.4 years Exclusion criteria: kidney failure defined by SCr > 132 μmol/L
Interventions	<ul style="list-style-type: none"> Study alternated 6 months with and 6 months without IFN-alpha therapy (2 x 10⁶ IU/d for a month, then every other day for 5 months). For those patients who started the trial with IFN-alpha, a 1 month wash-out period was included before the second half of the study
Outcomes	<ul style="list-style-type: none"> Presence and extent of purpura Serum glutamic pyruvic transaminase Renal involvement (proteinuria and SCr) Peripheral neuropathy (paraesthesia, motor disturbances, sensory-motor conduction velocity alterations) Cryocrit levels Total haemolytic complement activity Antinuclear, antimitochondrial, anti-smooth muscle antibodies Peripheral CD4+ and CD8+ lymphocytes
Notes	<ul style="list-style-type: none"> To be able to make the calculations, we only took into account the first period of the study before cross-over

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information to permit judgement
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgement

Ferri 1991 (Continued)

Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label study
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient information to permit judgement
Incomplete outcome data (attrition bias) All outcomes	High risk	In 'Table 2', there are 5 patients that drop-out and the authors did not present these data
Selective reporting (reporting bias)	High risk	In 'Methods Section' the authors specify that "renal and neurological involvement" and they did not report these outcomes later
Other bias	Low risk	Study appears free of other biases

Mazzaro 1995

Methods	<ul style="list-style-type: none"> Study design: parallel RCT Time frame: not reported Follow-up period: 18 months
Participants	<ul style="list-style-type: none"> Country: Italy Setting: multicentre Inclusion criteria: patients with mixed-cryoglobulinaemia diagnosis done by standard clinical and laboratory data and with resistance to previous treatment with corticosteroids and/or cytotoxic drugs Number: treatment group 1 (18); treatment group 2 (18) Mean age $\pm$ SD (years): treatment group 1 (53 ± 7); treatment group 2 (56 ± 10) Sex (F/M): 26/10 All of the patients showed cutaneous lesions (purpura) of variable severity. Polyneuropathy present in 14 (39%). Chronic liver disease in 24 (67%). All of the patients had been previously treated with corticosteroids and/or cytotoxic drugs and had become progressively resistant to such therapy. Exclusion criteria: homosexual people; drug-addiction; ethanol intake > 30 g
Interventions	<p>Treatment group 1</p> <ul style="list-style-type: none"> IFN-alpha-2b: 3 MU 3 times/week for 6 months <p>Treatment group 2</p> <ul style="list-style-type: none"> IFN-alpha-2b: 3 MU 3 times/week for one year
Outcomes	<ul style="list-style-type: none"> Response to the treatment <ul style="list-style-type: none"> Complete response: reduction of cryocrit to $> 20\%$ initial value, disappearance of all clinical manifestations of the disease (including purpura and neuropathy) and normalization of the Hb and liver function levels Partial response: disappearance of all clinical manifestations of the disease (including purpura and neuropathy), normalization of the Hb and liver function levels, but a reduction of cryocrit to $< 20\%$ Minor response: reduction of cryocrit $< 10\%$ initial value and disappearance of one or more (but not all) signs of vasculitis ALT Rheumatoid factor Purpura (score)

Mazzaro 1995 (Continued)

- HCV-RNA
- Adverse effects: thrombocytopenia, flu-like syndrome, depression, hypothyroidism

Notes

- The 2 IFN regimens have similar effect in terms of response to the treatment (group 1 versus group 2): complete response 28% versus 39%, partial response 50% both, minor response 22% versus 11%. Although differences were observed in terms of the duration of it: in group 1 89% of patients presented a relapse and only 11% maintained a long-term response (1 year) and in group 2 only 78% relapsed and long-term response was observed in 22%. The one-year therapy was linked to a higher number of side-effects (severe enough to cause the discontinuation of treatment in 2 cases) than the 6-month schedule.
- The IFN used was alpha 2b, new generation of IFN (PEG-IFN-alpha-2a) present a different profile

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information to permit judgement
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgement
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label study
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient information to permit judgement
Incomplete outcome data (attrition bias) All outcomes	Low risk	No exclusions or attrition reported
Selective reporting (reporting bias)	Low risk	Reported clearly the different outcomes
Other bias	Low risk	Study appears free of other biases

Mazzaro 2000
Methods

- Study design: parallel RCT
- Time frame: not reported
- Follow-up period: 18 month

Participants

- Country: Italy
- Setting: single centre
- Inclusion criteria: HCV cryoglobulinaemic GN diagnosed by kidney biopsy in stationary clinical phase of the disease
- Number: treatment group (7); control group (6)
- Mean age $\pm$ SD (years): treatment group (64 ± 12); control group (59 ± 11)
- Sex (F/M): treatment group (4/3); control group (4/2)
- All patients had previously been treated with corticosteroids and cyclophosphamide, and after remission of the acute phase, were randomised to one of the two treatments

Mazzaro 2000 (Continued)

	<ul style="list-style-type: none"> Exclusion criteria: homosexual people; history of intravenous drug or ethanol abuse
Interventions	<p>Treatment group</p> <ul style="list-style-type: none"> Lymphoblastoid IFN: 3 MU, 3 times/week for 6 months <p>Control group</p> <ul style="list-style-type: none"> Oral prednisone: 0.2 mg/kg/d for 6 months
Outcomes	<ul style="list-style-type: none"> Response to the therapy <ul style="list-style-type: none"> Complete response: decrease and/or return to normal of SCr, return to normal of proteinuria, disappearance of the cryocrit associated with the disappearance of all clinical manifestations of the disease (including purpura) Partial response: decrease of SCr and of proteinuria > 50%, disappearance of all signs of the disease, reduction of cryocrit of > 50% Minimal response: decrease of SCr, decrease of proteinuria < 50%, disappearance of one or more of the signs of the disease (but not all), and a reduction of the cryocrit ~50% No response: no modification or improvement ~10% of SCr, proteinuria, and cryocrit Purpura score Cryocrit level SCr Proteinuria C4 Rheumatoid factor HCV-RNA ALT
Notes	<ul style="list-style-type: none"> Both IFN and prednisone are associated with an improvement in clinical symptoms even in non-responders, with minor side-effects only in IFN group (fever, fatigue and a flu-like syndrome in most patients during the first two weeks) with one patient withdrawal (because thrombocytopenia). Prednisone was associated with a worsening in liver function tests. Only 1 case presented complete recovery and was from IFN group, indicating that complete remission from the disease can be obtained only through eradication of the virus. The type of IFN used was lymphoblastoid, new generation of IFN present a different profile.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information to permit judgement
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgement
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label study
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient information to permit judgement
Incomplete outcome data (attrition bias)	Low risk	Well specified. Quote: "All patients were followed for at least 12 months after the end of treatment".

Mazzaro 2000 (Continued)

All outcomes

Selective reporting (reporting bias)	Low risk	All outcomes reported
Other bias	Low risk	Study appears free of other biases

Misiani 1994

Methods	<ul style="list-style-type: none"> Study design: parallel RCT Time-frame: January 1991 to February 1992 Follow-up period: 24 to 48 weeks
Participants	<ul style="list-style-type: none"> Country: Italy Setting: single centre Inclusion criteria: HVC-associated type II cryoglobulinaemia Number: treatment group (27); control group (26) Median age, range (years): treatment group (62, 30 to 70); control group (63, 47 to 70) Sex (F/M): treatment group (23/4), control group (22/4) Duration of disease (months: median (range): treatment group (76, 12 to 228), control group (75, 9 to 226). Arthralgia: treatment group (19); control group (20) Kidney disease: treatment group (20); control group (20) Corticoid treatment: treatment group (22), control group (22) Exclusion criteria: secondary cryoglobulinaemia; hepatic failure (bilirubin > 4 mg/dL, albumin < 3 g/dL, Prothrombin time 3 seconds longer than normal); cytopenia; serious medical illness other than liver disease
Interventions	<p>Treatment group</p> <ul style="list-style-type: none"> IFN-alpha-2 (SC): 1.5 MU 3 times/week for a week and then 3 MU 3 times/week for 23 weeks <p>Control group</p> <ul style="list-style-type: none"> Previously prescribed treatments including: low-dose prednisone (≤ 0.2 mg/kg/d); diuretics; antihypertensive drugs and medications to treat associated diseases. The authors present this data in the article and there were no differences between groups
Outcomes	<ul style="list-style-type: none"> Cutaneous vasculitis (using a scoring system) SCr Proteinuria (data available given in protein-creatinine index (mg/mg), we estimated 24h proteinuria (mg/d) HCV-RNA Serum levels of anti-HVC antibodies Cryoglobulins IgM Rheumatoid factor Adverse events
Notes	<ul style="list-style-type: none"> The authors conclude that the beneficial effect of IFN is limited to patients in whom HCV RNA disappears from the serum (15/25 patients), whereas it worsened in those without response or in the control group IFN was well tolerated except for influenza-like illness, 2 cases discontinued because of atrial fibrillation and depression

Misiani 1994 (Continued)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer generated table
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgement
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label study
Blinding of outcome assessment (detection bias) All outcomes	High risk	There are also clinical variables that need blinding
Incomplete outcome data (attrition bias) All outcomes	High risk	Missing outcome data balanced in number across intervention groups, but the authors did not impute it using appropriate methods
Selective reporting (reporting bias)	Low risk	Data well specified
Other bias	Low risk	Study appears free of other biases

Pioltelli 1995

Methods	<ul style="list-style-type: none"> Study design: parallel RCT composed of three different studies <ol style="list-style-type: none"> Compared the efficacy of IFN and a steroid treatment Evaluated the effect of a low-antigen diet combined with drug treatment Estimated the need and usefulness of drug treatment in less severe stages of disease Time-frame: January 1991 to March 1993 Follow-up period: not reported
Participants	<ul style="list-style-type: none"> Country: Italy Setting: multicentre (number of centres not reported) Inclusion criteria: type II or III cryoglobulinaemia Number: treatment group 1 (28); treatment group 2 (28) Median age, range (years): treatment group 1 (62, 46 to 73); treatment group 2 (58, 29 to 73) Sex (F/M): treatment group 1 (20/8); treatment group 2 (21/7) Exclusion criteria: asymptomatic patients, severe disease (SCr > 176 µmol/L, acute nephritic syndrome, portal hypertension, hyperviscosity syndrome, ALT level five-fold the normal value, all patients with disorders which might be exacerbated by steroid or IFN treatment (diabetes mellitus, peptic ulcer, psychosis, congestive cardiomyopathy, coronary artery disease, malignant hypertension, glaucoma, ophthalmic herpes), lactating or pregnant women or malignancy
Interventions	Treatment group 1 <ul style="list-style-type: none"> Deflazacort: 0.1 mg/kg/d if mild disease or 0.2 mg/kg/d if moderate disease for at least 2 months and, if no improvement was obtained, was continued for a maximum of 4 months; after this time the dose was reduced to 0.1 mg/kg/d for mild disease and to 0.2 mg/kg/d for moderate disease

Pioltelli 1995 (Continued)

Treatment group 2

- IFN-alpha-2b: 3MU 3 times/week

Dose changes within 25% of the dose specified in the protocol schedule were allowed

Outcomes	<ul style="list-style-type: none"> • Absence of signs of disease lasting four months • Stage shift (from B to A, from A to B, or to stage C) and patient death. <ul style="list-style-type: none"> ◦ Clinical stages: Stage A (mild disease: purpura in distal segment of the limbs, weakness and joint pain); stage B (moderate disease: purpura in whole limbs or trunk, neurological involvement, SCr > 158.5 and < 176 µmol/L, ALT above the normal range) • C3 or C4 levels • Neurological impairment • Kidney impairment • Extent of purpura • Liver impairment • Karnofsky score • Side effects and compliance
Notes	<ul style="list-style-type: none"> • A total of 66 patients were enrolled (type II cryoglobulinaemia (51), type III cryoglobulinaemia (12)). They were stratified according to disease stage and underwent two independent randomisations <ul style="list-style-type: none"> ◦ Intervention 1: low antigen diet versus unrestrained diet ◦ Intervention 2: deflazacort versus IFN-alpha-2b • There was not available numerical data about Intervention 1 so it was not considered in the review • Authors did not find any difference between the effect of deflazacort and IFN, and the incidence of major toxic effects was higher in IFN group (25% of patients: ictus cerebri (1), refractory headache (1), refractory fever (1), refractory diarrhoea (1), anaphylaxis episode (1), impotentia coeundi (1), hypersomnia (1)) versus 14% in deflazacort group (femur fractures (2), diabetes mellitus (1), Cushing's syndrome (1))

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "The randomisation were performed centrally in a 1/1 ratio within balanced blocks of 8, stratified by the centre".
Allocation concealment (selection bias)	Low risk	Central randomisation
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label study
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Neurological impairment and extent of purpura was done by someone that is not well specified if was blinded or not.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data well specified
Selective reporting (reporting bias)	High risk	No data at the end of the study of these items: purpura, joint pain, weakness, kidney impairment, neurological impairment, liver impairment

Pioltelli 1995 (Continued)

Other bias	Low risk	Study appears free of other biases
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Sneller 2012

Methods	<ul style="list-style-type: none"> Study design: parallel RCT Time-frame: January 1991 to March 1993 Follow-up period: 12 months
Participants	<ul style="list-style-type: none"> Country: USA Setting: single centre Inclusion criteria: HVC-associated mixed cryoglobulinaemia patients in whom treatment with IFN and ribavirin failed to induced response or was not tolerated and showed by one or more than: cutaneous vasculitis, peripheral neuropathy and/or GN Number: treatment group (12); control group (12) Median age (years): treatment group (53); control group (51) Sex (F/M): treatment group (2/10); control group (4/8) Baseline activity (BVAS) were similar in both groups Exclusion criteria: change in immunosuppressive therapy 4 weeks of study entry; prior use of rituximab; lymphoma; severe kidney failure (CrCl < 30 mL/min); severe hepatic insufficiency (Child Pugh B or C); coinfection with VHB or HIV; liver transplantation; pregnancy; active systemic infection, or presence of potentially life-threatening vasculitis involving the central nervous system, heart, or gastrointestinal tract
Interventions	<p>Treatment group</p> <ul style="list-style-type: none"> Rituximab: 375 mg/m² days 1, 8, 15 and 22. Patients were allowed to continue any immunosuppressive therapy they were receiving prior randomisation. Premedication include 650 mg acetaminophen, diphenhydramine 50 mg and did not include steroids <p>Control group</p> <ul style="list-style-type: none"> Patients continued to take any immunosuppressive drugs (cyclophosphamide or methotrexate) or plasma exchange
Outcomes	<ul style="list-style-type: none"> Remission (defined as a BVAS of 0) at month 6 Duration of remission Occurrence of severe adverse effects
Notes	<ul style="list-style-type: none"> The trial was originally designed to enrol 30 patients but was stopped at a total enrolment of 24 patients when an interim analysis revealed that there was sufficient difference between groups The number of patients with remission at 6 months was significantly higher with rituximab treatment (P 0.001): 83.3% in rituximab group (95% CI 51.6 to 97.9) and 8.3% in control group (95% CI 2.0 to 38.6)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "randomly assigned in a 1 to 1"; method of randomisation not reported
Allocation concealment (selection bias)	Low risk	Central allocation: NIH Clinical Center Pharmacy performed the randomisation.

Sneller 2012 (Continued)

Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label study
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient information to permit judgement
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data is detailed. It is indicated that "analyses were performed on an intention-to-treat basis". The authors also stated that they present a "selected adverse effects" but they refer to each outcome defined in the methods section
Selective reporting (reporting bias)	Low risk	Data well specified
Other bias	Low risk	Study appears free of other biases

Stefanutti 2009

Methods	<ul style="list-style-type: none"> Study design: parallel RCT Time-frame: not reported Follow-up period: 24 weeks
Participants	<ul style="list-style-type: none"> Country: Italy Setting: single centre Inclusion criteria: HVC-associated mixed cryoglobulinaemia and at least 1 severe complication related to the pre-existing immunologic disorder Number: treatment group (9); control group (8) Mean age $\pm$ SD (years): treatment group (65.5 $\pm$ 14.0); control group (64.5 $\pm$ 13.4) Sex (F/M): treatment group (8/1); control group (6/2) Exclusion criteria: not reported
Interventions	<p>Patients received immunosuppressive therapy for 12 weeks, then were randomly assigned to two groups</p> <p>Treatment group</p> <ul style="list-style-type: none"> Immunoadsorption apheresis was done using Selesorb[®] with a plasma volume exchange of 45 mL/kg. The number of treatments for patient was between 24 and 35 Immunosuppressive therapy: included pegylated IFN 3 MU 3 times/week, cyclophosphamide 7.5 to 15 mg/kg (IV), CSA 2 to 5 mg/kg/d, ribavirin 10 to 15 mg/kg/d, or melphalan 0.1 to 0.2 mg/kg/d <p>Control group</p> <ul style="list-style-type: none"> Immunosuppressive therapy: included pegylated IFN 3 MU 3 times/week, cyclophosphamide 7.5 to 15 mg/kg (IV), CSA 2 to 5 mg/kg/d, ribavirin 10 to 15 mg/kg/d, or melphalan 0.1 to 0.2 mg/kg/d
Outcomes	<ul style="list-style-type: none"> Calculated clinical score using the following: cryocrit, renal function, neurologic system, skin, vascular system, and joints related signs and symptoms) calculated at baseline, at 12 and 24 weeks
Notes	<ul style="list-style-type: none"> Clinical score changes were more favourable in combined therapy with IA plus immunosuppressants compared to immunosuppressive treatment alone. Higher remission of severe clinical complications was found with combined therapy (80% versus 33%, P 0.05). It is mentioned in the article that "no adverse reactions or complications were noted".

Stefanutti 2009 (Continued)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "Randomly assigned"; method of randomisation not reported
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgement
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label study
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient information to permit judgement
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data well specified
Selective reporting (reporting bias)	Low risk	Data well specified
Other bias	Low risk	Although the immunoadsorption apheresis was interrupted before the scheduled therapy because of rapid relapse of symptomatology, the data of these patients is well reported

ALT- alanine aminotransferase; BVAS - Birmingham Vasculitis Activity Score; CMV - cytomegalovirus; CrCl - creatinine clearance; CSA - cyclosporine; F/M - female/male; IFN - interferon; IV - intravenous; Hb - haemoglobin; HCV - hepatitis C virus; HIV - human immunodeficiency virus; PCR - polymerase chain reaction; RCT - randomised controlled trial; SC - subcutaneous; SCr - serum creatinine; SD - standard deviation

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Anonymous 1989	Protocol of a RCT; no results identified
Candela 1994	Wrong population: mixed cryoglobulinaemia including different aetiologies with and without HCV
Ferri 1989	Wrong population: II and III type mixed cryoglobulinaemia including different aetiologies with and without HCV
Lauta 1995	Wrong population: II type mixed essential cryoglobulinaemia including different aetiologies with and without HCV
Vacca 1992	Wrong population: II and III type mixed essential cryoglobulinaemia including different aetiologies with and without HCV

HCV - hepatitis C virus; RCT - randomised controlled trial

DATA AND ANALYSES

Comparison 1. Rituximab versus no rituximab

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Death	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.1 One month	2	61	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
1.2 Six months	2	61	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
1.3 24 months	3	118	Risk Ratio (M-H, Random, 95% CI)	0.52 [0.10, 2.61]
2 Clinical manifestations	2		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.1 Active urinary sediment at 1 month	1	16	Risk Ratio (M-H, Random, 95% CI)	0.92 [0.51, 1.65]
2.2 Need for dialysis at 24 months	1	46	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
2.3 Skin vasculitis (purpura, cutaneous ulcers or others) at 1 month	1	57	Risk Ratio (M-H, Random, 95% CI)	2.59 [0.55, 12.27]
2.4 Skin vasculitis (purpura, cutaneous ulcers or others) at 18 and 24 months	2	78	Risk Ratio (M-H, Random, 95% CI)	0.57 [0.28, 1.16]
2.5 Skin vasculitis (purpura, cutaneous ulcers or others) at 36 months	1	37	Risk Ratio (M-H, Random, 95% CI)	0.68 [0.43, 1.08]
3 Laboratory findings	2		Mean Difference (IV, Random, 95% CI)	Subtotals only
3.1 Serum creatinine at 18 months [μmol/L]	1	37	Mean Difference (IV, Random, 95% CI)	-8.80 [-29.27, 11.67]
3.2 Serum creatinine at 36 months [μmol/L]	1	37	Mean Difference (IV, Random, 95% CI)	17.60 [-4.23, 39.43]
3.3 Proteinuria at 18 months [g/24 h]	1	3	Mean Difference (IV, Random, 95% CI)	-0.5 [-1.42, 0.42]
3.4 Proteinuria at 36 months [g/24 h]	1	3	Mean Difference (IV, Random, 95% CI)	0.24 [-0.84, 1.32]
3.5 Cryocrit at 12 months [%]	2	41	Mean Difference (IV, Random, 95% CI)	-2.01 [-10.29, 6.27]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.6 Serum HCV-RNA at 12 months [IU/mL]	1	17	Mean Difference (IV, Random, 95% CI)	-435182.0 [-1051224.79, 180860.79]
4 Adverse effects of the medication	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.1 Infusion reactions	3	118	Risk Ratio (M-H, Random, 95% CI)	4.33 [0.76, 24.75]
4.2 Discontinuation of the treatment due to adverse drug reactions	3	118	Risk Ratio (M-H, Random, 95% CI)	0.97 [0.22, 4.36]
4.3 Infection (pneumonia, urosepsis) at 6 months	1	24	Risk Ratio (M-H, Random, 95% CI)	0.50 [0.05, 4.81]
4.4 Infection (pneumonia, urosepsis) at 24 months	1	57	Risk Ratio (M-H, Random, 95% CI)	0.35 [0.04, 3.12]
4.5 Cardiovascular events (angina, myocardial infarction, heart failure) at 24 months	1	57	Risk Ratio (M-H, Random, 95% CI)	0.52 [0.10, 2.61]
4.6 Gastrointestinal bleeding	1	57	Risk Ratio (M-H, Random, 95% CI)	3.10 [0.13, 73.12]
4.7 Haemorrhagic alveolitis	1	57	Risk Ratio (M-H, Random, 95% CI)	0.34 [0.01, 8.12]
4.8 Leukopenia at 6 months	1	24	Risk Ratio (M-H, Random, 95% CI)	0.50 [0.05, 4.81]
4.9 Thrombocytopenia at 6 months	1	24	Risk Ratio (M-H, Random, 95% CI)	1.0 [0.07, 14.21]
5 Antiviral therapy failure or not indicated	3	118	Risk Ratio (M-H, Random, 95% CI)	1.21 [0.77, 1.90]

Analysis 1.1. Comparison 1 Rituximab versus no rituximab, Outcome 1 Death.

Study or subgroup	Rituximab n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.1.1 One month					
Sneller 2012	0/12	0/12			Not estimable
Dammacco 2010	0/22	0/15			Not estimable
Subtotal (95% CI)	34	27			Not estimable
Total events: 0 (Rituximab), 0 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					
<div> <div>Less with rituximab</div> <div>0.010.1110100</div> <div>Less with control</div> </div>

Analysis 1.2. Comparison 1 Rituximab versus no rituximab, Outcome 2 Clinical manifestations.

Analysis 1.3. Comparison 1 Rituximab versus no rituximab, Outcome 3 Laboratory findings.

Analysis 1.4. Comparison 1 Rituximab versus no rituximab, Outcome 4 Adverse effects of the medication.

Analysis 1.5. Comparison 1 Rituximab versus no rituximab, Outcome 5 Antiviral therapy failure or not indicated.

Comparison 2. Interferon versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Death	4		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.1 12 months	4	160	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
1.2 24 months	2	91	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
2 Clinical manifestations	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.1 Skin vasculitis (purpura, cutaneous ulcers or others) at 12 months	3	95	Risk Ratio (M-H, Random, 95% CI)	0.60 [0.36, 1.00]
3 Laboratory findings	4		Mean Difference (IV, Random, 95% CI)	Subtotals only
3.1 Serum creatinine at 18 months [μmol/L]	2	49	Mean Difference (IV, Random, 95% CI)	-30.32 [-80.59, 19.95]
3.2 Proteinuria at 18 months [g/24h]	2	49	Mean Difference (IV, Random, 95% CI)	-1.98 [-2.89, -1.07]
3.3 ALT or GPT at 6 months [UI/L]	2	39	Mean Difference (IV, Random, 95% CI)	-5.89 [-55.77, 43.99]
3.4 ALT or GPT at 18 months [UI/L]	2	49	Mean Difference (IV, Random, 95% CI)	-28.28 [-48.03, -8.54]
3.5 Rheumatoid factor activity at 6 months [UI/mL]	1	13	Mean Difference (IV, Random, 95% CI)	97.0 [-187.37, 381.37]
3.6 C4 at 18 months [mg/dL]	2	49	Mean Difference (IV, Random, 95% CI)	-0.04 [-2.74, 2.67]
3.7 IgM at 18 months [mg/dL]	2	52	Mean Difference (IV, Random, 95% CI)	-595.75 [-877.20, -314.30]
3.8 Cryocrit at 6 months [%]	2	39	Mean Difference (IV, Random, 95% CI)	-1.38 [-2.38, -0.38]
4 Adverse effects of the medication	5		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.1 Infusion reactions	1	65	Risk Ratio (M-H, Random, 95% CI)	27.82 [1.72, 449.18]
4.2 Discontinuations of the treatment due to adverse drug reactions	4	148	Risk Ratio (M-H, Random, 95% CI)	2.32 [0.91, 5.90]
5 Antiviral therapy failure or not indicated	2	78	Risk Ratio (M-H, Random, 95% CI)	0.59 [0.21, 1.64]

Analysis 2.1. Comparison 2 Interferon versus control, Outcome 1 Death.

Analysis 2.2. Comparison 2 Interferon versus control, Outcome 2 Clinical manifestations.

Analysis 2.3. Comparison 2 Interferon versus control, Outcome 3 Laboratory findings.

Analysis 2.4. Comparison 2 Interferon versus control, Outcome 4 Adverse effects of the medication.

Analysis 2.5. Comparison 2 Interferon versus control, Outcome 5 Antiviral therapy failure or not indicated.

Comparison 3. Interferon for 6 months versus 1 year

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Death at 24 months	1	36	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]

Analysis 3.1. Comparison 3 Interferon for 6 months versus 1 year, Outcome 1 Death at 24 months.

Study or subgroup	6 months n/N	1 year n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Mazzaro 1995	0/18	0/18			Not estimable

6 months 0.01 0.1 1 10 100 1 year

Study or subgroup	6 months n/N	1 year n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Total (95% CI)	18	18			Not estimable
Total events: 0 (6 months), 0 (1 year)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					
		6 months	0.01 0.1 1 10 100 1 year		

Comparison 4. Immunoabsorption apheresis versus immunosuppressive drug therapy

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Death at 24 months	1	17	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
2 Clinical manifestations	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected
2.1 Skin vasculitis (purpura, cutaneous ulcers or others) at 6 months	1		Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
2.2 Peripheral neuropathies at 6 months	1		Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
2.3 Peripheral joint arthralgia at 6 months	1		Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
3 Laboratory findings	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
3.1 Cryocrit [%]	1		Mean Difference (IV, Random, 95% CI)	0.0 [0.0, 0.0]
4 Adverse effects of the medication	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.1 Infusion reactions	1	17	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]

Analysis 4.1. Comparison 4 Immunoabsorption apheresis versus immunosuppressive drug therapy, Outcome 1 Death at 24 months.

Study or subgroup	Immunoabsorption n/N	Immunosuppressive drugs n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Stefanutti 2009	0/9	0/8			Not estimable
Total (95% CI)	9	8			Not estimable
Total events: 0 (Immunoabsorption), 0 (Immunosuppressive drugs)					
Heterogeneity: Not applicable					
	Immunoabsorption	0.01 0.1 1 10 100	Immunosuppressive drugs		

Study or subgroup	Immuno-adsorption n/N	Immunosup-pressive drugs n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Test for overall effect: Not applicable					
			0.01 0.1 1 10 100		
			Immunoabsorption		Immunosuppressive drugs

Analysis 4.2. Comparison 4 Immunoabsorption apheresis versus immunosuppressive drug therapy, Outcome 2 Clinical manifestations.

Study or subgroup	Immunoabsorption n/N	Immunosup-pressive drugs n/N	Risk Ratio M-H, Random, 95% CI	Risk Ratio M-H, Random, 95% CI
4.2.1 Skin vasculitis (purpura, cutaneous ulcers or others) at 6 months				
Stefanutti 2009	1/9	2/8		0.44[0.05,4.02]
4.2.2 Peripheral neuropathies at 6 months				
Stefanutti 2009	1/9	0/8		2.7[0.13,58.24]
4.2.3 Peripheral joint arthralgia at 6 months				
Stefanutti 2009	1/9	0/8		2.7[0.13,58.24]
			Favours immunoabsorption 0.01 0.1 1 10 100	Favours immunosup-pressive drugs

Analysis 4.3. Comparison 4 Immunoabsorption apheresis versus immunosuppressive drug therapy, Outcome 3 Laboratory findings.

Study or subgroup	Immunoabsorption		Immunosup-pressive drugs		Mean Difference Random, 95% CI	Mean Difference Random, 95% CI
	N	Mean(SD)	N	Mean(SD)		
4.3.1 Cryocrit [%]						
Stefanutti 2009	9	2.9 (1)	8	2.9 (2.5)		0.01[-1.86,1.88]
					Favours immunoabsorption -4 -2 0 2 4	Favours immunosup-pressive drugs

Analysis 4.4. Comparison 4 Immunoabsorption apheresis versus immunosuppressive drug therapy, Outcome 4 Adverse effects of the medication.

Study or subgroup	Immuno-adsorption n/N	Immunosup-pressive drugs n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
4.4.1 Infusion reactions					
Stefanutti 2009	0/9	0/8			Not estimable
Subtotal (95% CI)	9	8			Not estimable
Total events: 0 (Immunoabsorption), 0 (Immunosuppressive drugs)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					
			0.01 0.1 1 10 100		
			Favours [Immunoabsorption]		Favours [Immunosuppressive drugs]

ADDITIONAL TABLES

Table 1. Studies including new antiviral treatments for HCV-related mixed cryoglobulinaemia

Study ID	Study design	Patient profile	Objective	No. of patients	Treatment	Results	Adverse effects
Gragnani 2016	Non-randomised, non-controlled prospective study	HCV RNA (+) + cryoglobulinaemic syndrome with organ damage and B-cell lymphoproliferative syndrome	To assess the hepatovirological response, the clinical and immunological efficacy and the safety of using sofosbuvir-based direct-acting antiviral therapy in patients with HCV-associated mixed cryoglobulinaemia (according to the latest guidelines)	44	1) Sofosbuvir+ribavirin (18) 2) Sofosbuvir+simeprevir (12) ± ribavirin (6 of 12 patients) 3) Sofosbuvir+daclatasvir (4) ± ribavirin (1 patient) 4) Sofosbuvir+ledipasvir (10) ± ribavirin (3 patients)	Hepatovirological response - Undetectable HCV RNA negative rate 100% at week 4; 12SVR and 24SVR remained 100% negative - Decrease of ALT from 77.7 ± 10.3 IU/L at baseline to 27.3 ± 10.3 IU/L at 24SVR - Decrease of AST from 55.2 ± 60.4 IU/L at baseline to 22.6 ± 8.3 IU/L at 24SVR ($P < 0.001$) Clinical efficacy - Decrease of BVAS from 5.41 ± 3.53 at baseline to 1.27 ± 1.68 at 24SVR ($P < 0.001$) Immunological efficacy - Decrease of cryocrit level from $7.2 \pm 15.4\%$ at baseline to $1.8 \pm 5.1\%$ at 24SVR ($P < 0.001$)	Total: 26/44 (59%) Withdrawals: 1 patient withdrew ribavirin while continuing sofosbuvir+simeprevir Death: none Relapse: none Most frequent AE: Anaemia (13, all receiving ribavirin); fatigue (15); nausea (7)
Cornella 2015	Case series	Five patients with HVC RNA + with detectable cryoglobulins in plasma and symptomatic mixed cryoglobulinaemia Patient 1: bilateral foot neuropathy and purpura Patient 2: painful left foot	Review of one centre's experience in treating patients with mixed cryoglobulinaemia with new oral antiviral agents and to assess common factors associated with persistence of mixed cryoglobulinaemia despite SVR	5	Patient 1: PEG-IFN+ribavirin+boceprevir Patient 2: Firstly with rituximab (5 weeks); later with: PEG-IFN+ribavirin+telaprevir for 4 weeks; PEG-IFN+ribavirin for 12 weeks; and PEG-IFN+ribavirin+sofosbuvir for 15 weeks (telaprevir discontinued)	Patient 1: complete clearance of virus at week 8; complete clearance of cryoglobulins at week 28. Persistence of neuropathy Patient 2: SVR and no detectable cryoglobulins at month 6 after last triple therapy. Persistence of neuropathy. Patient 3: clearance of HCV at week 4 after PEG-IFN+ribavirin+telaprevir, with persistent cryoglobulins.	

Table 1. Studies including new antiviral treatments for HCV-related mixed cryoglobulinaemia (Continued)

		drop and purpura			for persistent viral load > 1000 IU/mL	Active MPGN and vasculitis after ending of previous treatment (responding to steroid therapy).	
		Patient 3: purpura and MPGN			Patient 3: PEG-IFN + ribavirin + telaprevir for 47 weeks	Patient 4: SVR with persistence of cryoglobulinaemia. Kidney function remained stable.	
		Patient 4: MPGN			Patient 4: PEG-IFN + ribavirin for 24 weeks; afterwards adding sofosbuvir until completing 12 weeks of triple therapy (total 36 weeks)	Patient 5: SVR 6 months after treatment without cryoglobulins. Rituximab at maintenance dose for lymphoma treatment	
		Patient 5: MPGN + low grade lymphoma			Patient 5: 2 cycles of rituximab: weekly infusions 375mg/m ² during 4 weeks in 2010 then two extra doses of rituximab in 2013		
					-PEG-IFN+ribavirin+sofosbuvir for 8 weeks in 2014		
Sise 2016	Retrospective case series	HCV RNA > 1000 IU/mL + circulating purpura + cutaneous ulcers, Raynaud's phenomenon, arthralgia, sicca syndrome, gastrointestinal vasculitis, neurologic involvement or renal involvement	Comparison of 2 historical cohorts: one treated with PEG-IFN and ribavirin and the other sofosbuvir+simeprevir (8/12) or sofosbuvir+ribavirin (4/12). Evaluation of 12SVR, relapses, clinical, immunological (cryoglobulins) and biochemical (AST, Hb) response and adverse effects;	22	1) PEG-IFN+ribavirin 2) IFN-free regimens 2a) Sofosbuvir 400 mg/24 h + simeprevir 150 mg/24h 2b) Sofosbuvir 400 mg/24 h + ribavirin (adjusted to kidney function)	PEG-IFN+ribavirin - SVR12: 1/10 IFN-free regimens - SVR12: 10/12 (95%) - ALT decreased from 42 U/L at baseline to 20 U/L after treatment - Cryoglobulin levels decreased from 1.5% (0.5% to 4%) at baseline to 0.5% (0% to 2%) after treatment - Decrease of proteinuria in all cases of kidney involvement (table IV)	Total PEG-IFN+ribavirin: 10/10 IFN-free regimens: 8/12 Withdrawals PEG-IFN+ribavirin: 5/10 IFN-free regimens: 1/12 (anxiety and insomnia) Deaths: none

Table 1. Studies including new antiviral treatments for HCV-related mixed cryoglobulinaemia (Continued)

			without statistical comparison between them				<p>Relapses</p> <p>PEG-IFN+rib-avirin: not specified</p> <p>IFN-free regimens: 2 (genotype 1</p> <p>sofosbuvir+simeprevir; genotype 4 sofosbuvir+ribavirin)</p>
Saadoun 2014	Open-label, non-controlled, prospective cohort study	Chronic active HCV infection with signs of mixed cryoglobulinaemia. All 23 patients had positive cryoglobulins in plasma at baseline or earlier	To analyse the safety and efficacy of Peg-IFN-alpha/rib-avirin/protease inhibitor combination in HCV-mixed cryoglobulinaemia	23	<p>Peg-IFN-alpha+rib-avirin</p> <p>a) + telaprevir (375 mg, 3 times/d for 12 weeks) for 48 weeks (15 patients)</p> <p>b) + boceprevir (800 mg, 3 times/d for 44 weeks) for 48 weeks (8 patients)</p>	<p>Complete clinical responders (improvement in all baseline clinical manifestations): 13 patients (56.5%) at week 24</p> <p>Virological response (i.e., HCV RNA negative) was of 69.6% at week 24 (P = 0.005).</p> <p>Cryoglobulin level: decreased from 0.44 to 0.06 g/L (P = 0.0006)</p> <p>C4 level: increased from 0.09 to 0.15 g/L (P = 0.045)</p> <p>No significant difference was found between the two treatment regimens</p>	<p>Total: 105</p> <p>Withdrawals: 8 patients (34.7%) (virological non-response (5); virological relapse (2); depression (1))</p> <p>Death: none</p> <p>Relapse: 1</p> <p>Most frequent AE:</p> <p>fatigue (87%); neutropenia (78.3%); thrombocytopenia (65.2%); infection (47.8%); pruritus (39.1%); depression (21.7%); nausea (21.7%)</p>
Saadoun 2016	Open-label, non-controlled, prospective cohort study	Active HCV infection and active mixed cryoglobulinaemia. Excluded non-	To evaluate safety and efficacy of an oral IFN-free regimen, sofosbuvir+ribavirin, in	24	Sofosbuvir (400 mg/d) + ribavirin (200 to 1400 mg/d) for 24 weeks	<p>Complete response (improvement of ALL the affected organs involved at baseline) at week 24: 21 (87.5%)</p>	<p>Total: 14/24 (54%)</p> <p>Withdrawals: 2 (8%)</p>

Table 1. Studies including new antiviral treatments for HCV-related mixed cryoglobulinaemia (Continued)

active mixed cryoglobulinaemia, HIV or HBV active infection and current decompensated cirrhosis	HCV-mixed cryoglobulinaemia	<i>“Rituximab was used in four cases, in addition to prednisone and plasmapheresis in two patients”</i>	<p>HCV RNA clearance at week 24: 22/24 (91.7%)</p> <p>SVR12: 74%</p> <p>Cryocrit: decrease from 0.35 g/L at baseline to 0 at 12 weeks after end-of-treatment.</p> <p>C4: increase from 0.1 g/L at baseline to 0.22 g/L at 12 week after end-of treatment</p> <p><i>“No difference of outcome was found in patients who received immunosuppressive treatment or not”</i></p>	<p>(hallucination and irritability (1); grade 4 anaemia (1))</p> <p>Death: 2 (severe pneumonia in the context of B cell lymphoma; pulmonary embolism in the context of hepatocellular carcinoma)</p> <p>Relapses: none</p> <p>Most frequent AE: (fatigue (25%); anaemia (25%); insomnia (21%); infection (17%); alopecia (8%))</p>
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12SVR - 12 week SVR; 24SVR - 24 week SVR; AE - adverse event; ALT - alanine aminotransferase; AST - aspartate aminotransferase; BVAS - Birmingham Vasculitis Activity Score; Hb - haemoglobin; HBV - hepatitis B virus; HCV - hepatitis C virus; HIV - human immunodeficiency virus; IFN - interferon; MPGN - membranoproliferative glomerulonephritis; PEG - pegylated; SVR - sustained viral response

APPENDICES

Appendix 1. Electronic search strategies

Database	Search terms
CENTRAL	1. cryoglobulin*emi*:ti.ab.kw
MEDLINE	1. Cryoglobulinemia/ 2. Cryoglobulin?emi*.tw. 3. or/1-2 4. exp Hepatitis C/ 5. Hepacivirus/ 6. hepatitis C.tw. 7. hep c.tw. 8. hcv.tw. 9. or/4-7 10.3 and 9
EMBASE	1. Cryoglobulinemia/ 2. cryoglobulin?emi*.tw. 3. or/1-2 4. Hepatitis C/ 5. Hepatitis C virus/ 6. hepatitis C.tw. 7. hep c.tw. 8. hcv.tw. 9. or/4-8 10.and/3,9

Appendix 2. Risk of bias assessment tool

Potential source of bias	Assessment criteria
Random sequence generation Selection bias (biased allocation to interventions) due to inadequate generation of a randomised sequence	<p><i>Low risk of bias:</i> Random number table; computer random number generator; coin tossing; shuffling cards or envelopes; throwing dice; drawing of lots; minimization (minimization may be implemented without a random element, and this is considered to be equivalent to being random).</p> <p><i>High risk of bias:</i> Sequence generated by odd or even date of birth; date (or day) of admission; sequence generated by hospital or clinic record number; allocation by judgement of the clinician; by preference of the participant; based on the results of a laboratory test or a series of tests; by availability of the intervention.</p> <p><i>Unclear:</i> Insufficient information about the sequence generation process to permit judgement.</p>
Allocation concealment Selection bias (biased allocation to interventions) due to	<p><i>Low risk of bias:</i> Randomisation method described that would not allow investigator/participant to know or influence intervention group before eligible participant entered in the study (e.g. central allocation, including telephone, web-based, and pharmacy-controlled, randomisation; sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes).</p>

(Continued)

inadequate concealment of allocations prior to assignment

High risk of bias: Using an open random allocation schedule (e.g. a list of random numbers); assignment envelopes were used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or not sequentially numbered); alternation or rotation; date of birth; case record number; any other explicitly unconcealed procedure.

Unclear: Randomisation stated but no information on method used is available.

Blinding of participants and personnel

Performance bias due to knowledge of the allocated interventions by participants and personnel during the study

Low risk of bias: No blinding or incomplete blinding, but the review authors judge that the outcome is not likely to be influenced by lack of blinding; blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken.

High risk of bias: No blinding or incomplete blinding, and the outcome is likely to be influenced by lack of blinding; blinding of key study participants and personnel attempted, but likely that the blinding could have been broken, and the outcome is likely to be influenced by lack of blinding.

Unclear: Insufficient information to permit judgement

Blinding of outcome assessment

Detection bias due to knowledge of the allocated interventions by outcome assessors.

Low risk of bias: No blinding of outcome assessment, but the review authors judge that the outcome measurement is not likely to be influenced by lack of blinding; blinding of outcome assessment ensured, and unlikely that the blinding could have been broken.

High risk of bias: No blinding of outcome assessment, and the outcome measurement is likely to be influenced by lack of blinding; blinding of outcome assessment, but likely that the blinding could have been broken, and the outcome measurement is likely to be influenced by lack of blinding.

Unclear: Insufficient information to permit judgement

Incomplete outcome data

Attrition bias due to amount, nature or handling of incomplete outcome data.

Low risk of bias: No missing outcome data; reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; missing data have been imputed using appropriate methods.

High risk of bias: Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; 'as-treated' analysis done with substantial departure of the intervention received from that assigned at randomisation; potentially inappropriate application of simple imputation.

Unclear: Insufficient information to permit judgement

Selective reporting

Reporting bias due to selective outcome reporting

Low risk of bias: The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way; the study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon).

High risk of bias: Not all of the study's pre-specified primary outcomes have been reported; one or more primary outcomes is reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified; one or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); one or more outcomes of interest in the review are reported incompletely so that they can-

(Continued)

not be entered in a meta-analysis; the study report fails to include results for a key outcome that would be expected to have been reported for such a study.

Unclear: Insufficient information to permit judgement

Other bias

Low risk of bias: The study appears to be free of other sources of bias.

Bias due to problems not covered elsewhere in the table

High risk of bias: Had a potential source of bias related to the specific study design used; stopped early due to some data-dependent process (including a formal-stopping rule); had extreme baseline imbalance; has been claimed to have been fraudulent; had some other problem.

Unclear: Insufficient information to assess whether an important risk of bias exists; insufficient rationale or evidence that an identified problem will introduce bias.

CONTRIBUTIONS OF AUTHORS

1. Draft the protocol: NM, ER, CB, JP, MS
2. Study selection: NM, MS
3. Extract data from studies: NM, AF, ER, MS
4. Enter data into RevMan: NM
5. Carry out the analysis: NM
6. Interpret the analysis: NM, MS
7. Draft the final review: All authors
8. Disagreement resolution: JP
9. Update the review: NM, MS

DECLARATIONS OF INTEREST

- Nuria Montero: none known
- Alexandre Fava: none known
- Eva Rodriguez: none known
- Clara Barrios: none known
- Josep M Cruzado: none known
- Julio Pascual: has advisory board and/or clinical trial involvement with Novartis, Astellas, Roche, Wyeth and Genzyme, and has also been an invited speaker at national and international meetings sponsored by Novartis and Astellas
- Maria Jose Soler: none known

SOURCES OF SUPPORT

Internal sources

- None, Other.

External sources

- None, Other.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Although initially we did not want to include cluster trials, at the end we finally included one. Although it was not initially planned and consequently, it was not initially in the protocol, we decided to evaluate activity outcomes as they were the main comparison in the majority of included studies.

INDEX TERMS

Medical Subject Headings (MeSH)

Antiviral Agents [*therapeutic use]; Blood Component Removal [methods]; Cryoglobulinemia [*therapy] [virology]; Hepacivirus; Hepatitis C [complications] [*drug therapy]; Immunologic Factors [therapeutic use]; Immunosorbent Techniques; Immunosuppressive Agents [therapeutic use]; Interferons [therapeutic use]; Randomized Controlled Trials as Topic; Rituximab [therapeutic use]; Skin Diseases [*therapy] [virology]; Vasculitis [etiology] [*therapy]

MeSH check words

Humans