bacteria, but also fungi, viruses, and parasites. For each type of pathogen multiple species or subgroups exist for which specific binding ligands need to be provided. This inevitably also means that it is necessary to diagnose the pathogen causing the infection before pathogen removal by apheresis can be performed. Since immediate treatment is essential in sepsis, diagnosis of the infecting pathogen may cause an unacceptable delay.

Summary

It is an object of the present disclosure to provide a method for removing pathogens from blood or from a blood component and to provide methods of treatment of bacterial infections in the blood, in particular of sepsis, by apheresis that overcome the disadvantages of the currently known methods.

Disclosed is a method for the removal of a pathogen from blood or a blood product comprising red blood cells, said method comprising:

- separating said blood or blood product into at least two components, a first component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets and at least a second component comprising WBCs and platelets and substantially free of RBCs;
- contacting said first component with a filter comprising a non-specific RBC adhesion molecule binder thereby depleting said first component of RBCs that have bound at least one pathogen;
- optionally combining said first component that is depleted of RBCs that have bound at least one pathogen and said at least second component.

Further disclosed is a method for the removal of a pathogen from blood of a patient in need thereof comprising:

- withdrawing blood from said patient;
- separating said blood into at least two blood components, a first component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets and at least a second component comprising WBCs and platelets and substantially free of RBCs;
- contacting said first component with a filter comprising a non-specific RBC adhesion molecule binder thereby depleting said first component of RBCs that have bound at least one pathogen;
- optionally combining said first component that is depleted of RBCs that have bound at least one pathogen and said at least second component;

- returning said first component that is depleted of RBCs that have bound at least one pathogen and said at least second component to said patient.

In one aspect, there is provided an *in vitro* method for the removal of a pathogen selected from bacteria, fungi and/or viruses from blood or a blood product comprising red blood cells, said method comprising:

- separating said blood or blood product into at least two components, a first component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets and a second component comprising WBCs and platelets and substantially free of RBCs;
- contacting said first component with a filter comprising a non-specific RBC adhesion molecule binder thereby depleting said first component of RBCs that have bound at least one pathogen, wherein said non-specific RBC adhesion molecule binder is a polymer or a mixture of two or more polymers;
- combining said first component that is depleted of RBCs that have bound at least one pathogen and said second component.

In another aspect, there is provided an *ex vivo* method for the removal of a pathogen selected from bacteria, fungi and/or viruses from blood of a patient in need thereof, preferably for the treatment of sepsis, comprising:

- separating blood withdrawn from said patient into at least two blood components, a first component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets and a second component comprising WBCs and platelets and substantially free of RBCs;
- contacting said first component with a filter comprising a non-specific RBC adhesion molecule binder thereby depleting said first component of RBCs that have bound at least one pathogen, wherein said non-specific RBC adhesion molecule binder is a polymer or a mixture of two or more polymers;
- combining said first component that is depleted of RBCs that have bound at least one pathogen and said second component, wherein said first component that is depleted of RBCs that have bound at least one pathogen and said second component are to be returned to said patient.

Disclosed is a non-specific RBC adhesion molecule binder for use in a treatment of removing a pathogen from blood of a subject, said treatment comprising contacting a blood component comprising red blood cells and substantially free of white blood cells (WBCs) and platelets with a filter comprising

said non-specific RBC adhesion molecule binder thereby removing RBCs that have bound at least one pathogen from said blood component.

Disclosed is a non-specific red blood cell (RBC) adhesion molecule binder for use in a treatment of removing a pathogen from blood of a subject, said treatment comprising contacting a blood component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets with a filter comprising said non-specific RBC adhesion molecule binder to allow binding of RBCs that have bound at least one pathogen to said non-specific RBC adhesion molecule binder, thereby removing said RBCs that have bound at least one pathogen from said blood component.

Disclosed is a use of a non-specific RBC adhesion molecule binder in the manufacture of a filter comprising said non-specific RBC adhesion molecule binder for a treatment of removing a pathogen from blood of a subject, said treatment comprising contacting a blood component comprising RBCs, which blood component is substantially free of WBCs and platelets, with a filter comprising said non-specific RBC adhesion molecule binder thereby removing RBCs that have bound at least one pathogen from said blood component.

In another aspect, there is provided use of a non-specific red blood cell (RBC) adhesion molecule binder in the manufacture of a support, preferably a filter, for the treatment of removing a pathogen selected from bacteria, fungi and/or viruses from blood of a subject, preferably in the treatment of sepsis or a developing sepsis, said treatment comprising contacting a blood component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets with a filter comprising said non-specific RBC adhesion molecule binder to allow binding of RBCs that have bound at least one pathogen to said non-specific RBC adhesion molecule binder, thereby removing said RBCs that have bound at least one pathogen from said blood component and returning said blood component that is depleted of RBCs that have bound at least one pathogen and a second blood component comprising WBCs and platelets and substantially free of RBCs to said subject, wherein said non-specific RBC adhesion molecule binder is a polymer or a mixture of two or more polymers.

In another aspect, there is provided use of a support comprising a non-specific red blood cell (RBC) adhesion molecule binder for *in vitro* removal of pathogens from a blood component comprising RBCs and substantially free of WBCs and platelets, wherein said non-specific RBC adhesion molecule binder is a polymer or a mixture of two or more polymers.

Disclosed is a method for detecting pathogens in a sample of blood or of a blood product comprising red blood cells (RBCs), the method comprising:

i) contacting said sample with a support comprising a non-specific RBC adhesion molecule binder to allow binding of RBCs that have bound at least one pathogen to said binder;

ii) detecting the presence of RBCs that have bound at least one pathogen.

In another aspect, there is provided a method for detecting pathogens selected from bacteria, fungi and/or viruses in a sample of blood or of a blood product comprising red blood cells (RBCs), the method comprising:

i) contacting said sample with a support comprising a non-specific RBC adhesion molecule binder, wherein said non-specific RBC adhesion molecule binder is a polymer or mixture of two or more polymers, to allow binding of RBCs that have bound at least one pathogen to said binder;

ii) detecting the presence of RBCs that have bound at least one pathogen,

preferably further comprising separating said sample into at least two components prior to step i), a first component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets and a second component comprising WBCs and platelets and substantially free of RBCs, wherein step i) comprises contacting said first component of said sample with a support comprising said non-specific RBC adhesion molecule binder to allow binding of said RBCs that have bound at least one pathogen to said binder,

optionally further comprising isolating RBCs that are bound to said support from said sample, preferably by removing cells and other components in said sample that have not bound to said binder, and/or

further comprising lysing said RBCs that are bound to said support following said isolation step, and/or

further comprising determining at least one characteristic of said pathogens if RBCs that have bound at least one pathogen are detected in said sample, wherein said characteristic is selected from the group consisting of the type of pathogen, gram-positivity or gram-negativity if said pathogen is a bacterium, drug or antibiotic resistance of the pathogens, and the identity of the pathogens.

Disclosed is a method for identifying pathogens, such as sepsis-causing pathogens or infection-causing pathogens, in a

sample of blood or of a blood product comprising red blood cells (RBCs), the method comprising:

i) contacting said sample with a support comprising a non-specific RBC adhesion molecule binder to allow binding of RBCs that have bound at least one pathogen to said binder;

ii) isolating RBCs that are bound to said support from said sample, preferably by removing cells and other components in said sample that have not bound to said binder;

iii) identifying the pathogen that is bound to said RBCs bound to said support.

In another aspect, there is provided a method for identifying pathogens selected from bacteria, fungi and/or viruses in a sample of blood or of a blood product comprising red blood cells (RBCs), the method comprising:

i) contacting said sample with a support comprising a non-specific RBC adhesion molecule binder, wherein said non-specific RBC adhesion molecule binder is a polymer or mixture of two or more polymers, to allow binding of RBCs that have bound at least one pathogen to said binder;

ii) isolating RBCs that are bound to said support from said sample, preferably by removing cells and other components in said sample that have not bound to said binder;

iii) identifying the pathogen that is bound to said RBCs bound to said support.

In a still further aspect, there is provided a method for diagnosing an infection in the blood of a patient, the method comprising detecting pathogens and/or identifying pathogens in a sample of blood or a blood product comprising red blood cells (RBCs) of said patient with a method as described herein.

Disclosed is a method for isolating and/or enriching pathogens from a sample of blood or of a blood product comprising red blood cells (RBCs), the method comprising:

i) contacting said sample with a support comprising a non-specific RBC adhesion molecule binder to allow binding of RBCs that have bound at least one pathogen to said binder;

ii) isolating RBCs that are bound to said support from said sample, preferably by removing cells and other components in said sample that have not bound to said binder.

In another aspect, there is provided a method for isolating and/or enriching pathogens selected from bacteria, fungi and/or viruses from a sample of blood or of a blood product comprising red blood cells (RBCs), the method comprising:

i) contacting said sample with a support comprising a non-specific RBC adhesion molecule binder, wherein said non-specific RBC adhesion molecule binder is a polymer or mixture of two or more polymers, to allow binding of RBCs that have bound at least one pathogen to said binder;

ii) isolating RBCs that are bound to said support from said sample, preferably by removing cells and other components in said sample that have not bound to said binder.

Disclosed is a method for determining susceptibility for antibiotics of a pathogen, the method comprising,

- detecting pathogens and/or identifying pathogens in a sample of blood or a blood product comprising red blood cells (RBCs) with a method according to the disclosure
- isolating RBCs that are bound to said support from said sample;
- contacting said isolated RBCs that are bound to said support with one or more antibiotic agents; and
- determining the growth of a functional activity of the pathogen.

In another aspect, there is provided a method for determining susceptibility for antibiotics of a pathogen selected from bacteria, fungi and/or viruses, the method comprising:

- detecting pathogens selected from bacteria, fungi and/or viruses and/or identifying pathogens selected from bacteria, fungi and/or viruses in a sample of blood or a blood product comprising red blood cells (RBCs) with a method as described herein;
- isolating RBCs that are bound to said support from said sample;
- contacting said isolated RBCs that are bound to said support with one or more antibiotic agents; and
- determining the growth of a functional activity of the pathogen.

Disclosed is a method for determining the presence of pathogens in a whole blood sample of an individual suffering from sepsis, suspected of suffering from sepsis or at risk of developing sepsis, the method

comprising detecting the presence of red blood cells (RBCs) that have bound at least one pathogen using cytometry or microscopy.

Further disclosed is a system for removing a pathogen from blood or a blood product, wherein said system comprises a separation device for separating said blood or said blood product into at least a first component comprising red blood cells (RBCs) and being substantially free of white blood cells (WBCs) and platelets and at least a second component comprising white blood cells (WBCs) and platelets and being substantially free of red blood cells (RBCs), and wherein said system further comprises a filter device for depleting said first component of RBCs that have bound at least one pathogen, said filter device comprising a non-specific RBC adhesion molecule binder.

In another aspect, there is provided a system (1) for removing a pathogen selected from bacteria, fungi and/or viruses from blood or a blood product, comprising:

- a separation device (2), preferably comprising a centrifuge, for separating said blood or said blood product into at least a first component comprising red blood cells (RBCs) and being substantially free of white blood cells (WBCs) and platelets and a second component comprising white blood cells (WBCs) and platelets and being substantially free of red blood cells (RBCs);

- a filter device (3) for depleting said first component substantially of RBCs that have bound at least one pathogen, said filter device comprising a non-specific RBC adhesion molecule binder, wherein said non-specific RBC adhesion molecule binder is a polymer or a mixture of two or more polymers;

- a conduit (4) for conducting said first component from the separation device (2), e.g. from a first outlet thereof (2b), to the filter device (3), e.g. to an inlet thereof (3a);

- an outlet conduit (10) for conducting the filtered first component, which is substantially depleted of RBCs that have bound at least one pathogen, away from said filter device (3), wherein the system (1) is arranged to feed the filtered first component, which is substantially depleted of RBCs that have bound at least one pathogen, to a subject (9) and/or to an administering device (6b) for administering at least said filtered first component to a subject (9).

In another aspect, there is provided an apparatus for removing a pathogen selected from bacteria, fungi and/or viruses from blood or a blood product comprising a housing (15) and a system (1) as described herein.

Disclosed is a pathogen bound RBC filter device for use in such system, wherein said pathogen bound RBC filter device is arranged for depleting a blood

product of RBCs that have bound at least one pathogen, said filter device comprising a non-specific RBC adhesion molecule binder.

Detailed description

The invention relates to the detection and/or removal of pathogens from blood or from a blood component by targeting red blood cells (RBCs). The present inventors found that once RBCs have contacted and have bound a pathogen in the blood stream, the activation of adhesion molecules on the surface of these RBCs is increased. Without wishing to be bound by theory, it is believed that this mechanism contributes to clearance of pathogens from the blood by phagocytic monocytes and macrophages in the spleen and the liver. As described herein before, pathogens in the blood are quickly opsonized by complement fragments that bind to molecules on the surface of the pathogens. In humans and other primates RBCs bind complement opsonized pathogens and transport the pathogens to the liver and spleen. Here the pathogens are transferred from the RBCs to phagocytic cells whereby the RBCs remain intact and viable. The pathogens are subsequently taken up by phagocytic cells and degraded. Before the present invention it was believed that transfer of pathogens from RBCs to phagocytic cells is initiated once the phagocytic cells recognize the complement fragments of opsonized pathogens. However, the present invention demonstrates that RBCs change their phenotype after they bind complement opsonized pathogens via complement receptor CR1. More specifically the adhesion molecules on the cell surface of the RBCs are

Claims

1. An *in vitro* method for the removal of a pathogen selected from bacteria, fungi and/or viruses from blood or a blood product comprising red blood cells, said method comprising:

- separating said blood or blood product into at least two components, a first component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets and a second component comprising WBCs and platelets and substantially free of RBCs;

- contacting said first component with a filter comprising a non-specific RBC adhesion molecule binder thereby depleting said first component of RBCs that have bound at least one pathogen, wherein said non-specific RBC adhesion molecule binder is a polymer or a mixture of two or more polymers;

- combining said first component that is depleted of RBCs that have bound at least one pathogen and said second component.

2. Method according to claim 1, further comprising storing said first component that is depleted of RBCs that have bound at least one pathogen and said second component.

3. An *ex vivo* method for the removal of a pathogen selected from bacteria, fungi and/or viruses from blood of a patient in need thereof, preferably for the treatment of sepsis, comprising:

- separating blood withdrawn from said patient into at least two blood components, a first component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets and a second component comprising WBCs and platelets and substantially free of RBCs;

- contacting said first component with a filter comprising a non-specific RBC adhesion molecule binder thereby depleting said first component of RBCs

that have bound at least one pathogen, wherein said non-specific RBC adhesion molecule binder is a polymer or a mixture of two or more polymers;

- combining said first component that is depleted of RBCs that have bound at least one pathogen and said second component, wherein

said first component that is depleted of RBCs that have bound at least one pathogen and said second component are to be returned to said patient.

4. Use of a non-specific red blood cell (RBC) adhesion molecule binder in the manufacture of a support, preferably a filter, for the treatment of removing a pathogen selected from bacteria, fungi and/or viruses from blood of a subject, preferably in the treatment of sepsis or a developing sepsis, said treatment comprising contacting a blood component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets with a filter comprising said non-specific RBC adhesion molecule binder to allow binding of RBCs that have bound at least one pathogen to said non-specific RBC adhesion molecule binder, thereby removing said RBCs that have bound at least one pathogen from said blood component and returning said blood component that is depleted of RBCs that have bound at least one pathogen and a second blood component comprising WBCs and platelets and substantially free of RBCs to said subject,

wherein said non-specific RBC adhesion molecule binder is a polymer or a mixture of two or more polymers.

5. A use according to claim 4, wherein said treatment comprises separating said blood into at least two blood components, a first component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets and a second component comprising WBCs and platelets and substantially free of RBCs.

6. Use of a support comprising a non-specific red blood cell (RBC) adhesion molecule binder for in vitro removal of pathogens from a blood component comprising RBCs and substantially free of WBCs and platelets, wherein said non-specific RBC adhesion molecule binder is a polymer or a mixture of two or more polymers.

7. A system (1) for removing a pathogen selected from bacteria, fungi and/or viruses from blood or a blood product, comprising:

- a separation device (2), preferably comprising a centrifuge, for separating said blood or said blood product into at least a first component comprising red blood cells (RBCs) and being substantially free of white blood cells (WBCs) and platelets and a second component comprising white blood cells (WBCs) and platelets and being substantially free of red blood cells (RBCs);

- a filter device (3) for depleting said first component substantially of RBCs that have bound at least one pathogen, said filter device comprising a non-specific RBC adhesion molecule binder, wherein said non-specific RBC adhesion molecule binder is a polymer or a mixture of two or more polymers;

- a conduit (4) for conducting said first component from the separation device (2), e.g. from a first outlet thereof (2b), to the filter device (3), e.g. to an inlet thereof (3a);

- an outlet conduit (10) for conducting the filtered first component, which is substantially depleted of RBCs that have bound at least one pathogen, away from said filter device (3), wherein the system (1) is arranged to feed the filtered first component, which is substantially depleted of RBCs that have bound at least one pathogen, to a subject (9) and/or to an administering device (6b) for administering at least said filtered first component to a subject (9) .

8. A system (1) according to claim 7 further comprising:

- a second component outlet conduit (11) for conducting the separated second component away from the separation device (2), said second component outlet conduit (11) being in fluid connection with a second outlet (2c) of the separation device (2) for letting the second component out of the separation device (2),

wherein the system (1) is arranged to put the filtered first component and at least part of the second component of the blood or blood product together in order to obtain a blood or blood product substantially free of pathogens, or at least substantially free of RBC bound pathogens,

preferably wherein the outlet conduit (10) for conducting the filtered first component away from the filter device (3) merges with the second component outlet conduit (11), such that the filtered first component and the second component can join, and can preferably mix.

9. A system (1) according to claim 8, further comprising a mixing chamber (12) or another mixing unit (12) for mixing said second component with said filtered first component.

10. A system (1) according to claim 8 or 9, further comprising an anticoagulant supply unit (8) for adding an anticoagulant to the blood or the blood product, wherein said anticoagulant supply unit (8) is preferably situated upstream of the separation device (2), and more preferably is arranged for feeding the anticoagulant into a supply conduit (5) for supplying the blood or blood product to the separation device (2).

11. An apparatus for removing a pathogen selected from bacteria, fungi and/or viruses from blood or a blood product comprising a housing (15) and a system (1) according to any one of claims 8-10.

12. A method for detecting pathogens selected from bacteria, fungi and/or viruses in a sample of blood or of a blood product comprising red blood cells (RBCs), the method comprising:

i) contacting said sample with a support comprising a non-specific RBC adhesion molecule binder, wherein said non-specific RBC adhesion molecule binder is a polymer or mixture of two or more polymers, to allow binding of RBCs that have bound at least one pathogen to said binder;

ii) detecting the presence of RBCs that have bound at least one pathogen, preferably further comprising separating said sample into at least two components prior to step i), a first component comprising red blood cells (RBCs) and substantially free of white blood cells (WBCs) and platelets and a second component comprising WBCs and platelets and substantially free of RBCs, wherein step i) comprises contacting said first component of said sample with a support comprising said non-specific RBC adhesion molecule binder to allow binding of said RBCs that have bound at least one pathogen to said binder,

optionally further comprising isolating RBCs that are bound to said support from said sample, preferably by removing cells and other components in said sample that have not bound to said binder, and/or

further comprising lysing said RBCs that are bound to said support following said isolation step, and/or

further comprising determining at least one characteristic of said pathogens if RBCs that have bound at least one pathogen are detected in said sample, wherein said characteristic is selected from the group consisting of the type of pathogen, gram-positivity or gram-negativity if said pathogen is a bacterium, drug or antibiotic resistance of the pathogens, and the identity of the pathogens.

13. The method according to claim 11, wherein said support comprises beads coated with said non-specific RBC adhesion molecule binder, preferably a polymer or mixture of two or more polymers.

14. A method for identifying pathogens selected from bacteria, fungi and/or viruses in a sample of blood or of a blood product comprising red blood cells (RBCs), the method comprising:

i) contacting said sample with a support comprising a non-specific RBC adhesion molecule binder, wherein said non-specific RBC adhesion molecule binder is a polymer or mixture of two or more polymers, to allow binding of RBCs that have bound at least one pathogen to said binder;

ii) isolating RBCs that are bound to said support from said sample, preferably by removing cells and other components in said sample that have not bound to said binder;

iii) identifying the pathogen that is bound to said RBCs bound to said support.

15. A method for isolating and/or enriching pathogens selected from bacteria, fungi and/or viruses from a sample of blood or of a blood product comprising red blood cells (RBCs), the method comprising:

i) contacting said sample with a support comprising a non-specific RBC adhesion molecule binder, wherein said non-specific RBC adhesion molecule binder is a polymer or mixture of two or more polymers, to allow binding of RBCs that have bound at least one pathogen to said binder;

ii) isolating RBCs that are bound to said support from said sample, preferably by removing cells and other components in said sample that have not bound to said binder.

16. A method for determining susceptibility for antibiotics of a pathogen selected from bacteria, fungi and/or viruses, the method comprising:

- detecting pathogens selected from bacteria, fungi and/or viruses and/or identifying pathogens selected from bacteria, fungi and/or viruses in a sample of blood or a blood product comprising red blood cells (RBCs) with a method according to any one of claims 11-14;

- isolating RBCs that are bound to said support from said sample;

- contacting said isolated RBCs that are bound to said support with one or more antibiotic agents; and

- determining the growth of a functional activity of the pathogen.

17. A method for diagnosing an infection in the blood of a patient, the method comprising detecting pathogens and/or identifying pathogens in a sample of blood or a blood product comprising red blood cells (RBCs) of said patient with a method according to any one of claims 11-14.