A comparative analysis of four diagnostic methods for dengue infection, namely virus isolation, viral RNA detection, dengue specific IgM detection and NS1 antigen detection revealed that NS1 antigen detection had the highest sensitivity rate compared to the other three methods (Kumarasamy et al. 2007). Alcon *et al.* 2002 have described an ELISA for NS1 detection and demonstrated that NS1 is present at high levels in patient sera during primary and secondary infection. NS1 is detectable during the whole clinical phase of illness and can be detected in the first few days of infection (as early as the first day of fever). Falconar and Young, 1991 have described the production of dimer-specific and dengue virus group cross-reactive mouse monoclonal antibodies to DEN2 virus NS1 and the use of certain of these antibodies in an ELISA for NS1 (Young et al. 2000). High levels of NS1 were found in acute phase sera, but not in convalescent phase sera, from some of the patients with serologically confirmed DEN2 virus secondary infection. PCT Patent Application WO 00/75665 describes a method for detecting NS1 protein in the hexameric form, and the selection of antibodies directed against the NS1 secreted hexameric form, together with the use of such antibodies in the early detection of flavivirus infection.

The problem underlying the invention therefore is to provide new markers of dengue infection that have an added diagnostic and prognostic value compared to the commonly used NS1 marker, and more particularly to allow for better medical care of patients at risk to develop a severe dengue, and to decrease mortality rates.

SUMMARY

The problem is solved by the current invention as specified in the claims.

In one aspect, the present disclosure refers to an *in vitro* method of diagnostic, prognostic and monitoring of a flaviviral infection, comprising detecting the presence or level of a complex formed by dimers of a flaviviral non-structural protein 1 (NS1) bound at the surface of plasma High Density Lipoprotein (HDL) particles in a blood sample obtained from a subject, wherein presence or level of said complex in the blood sample is indicative of a flaviviral infection. In another aspect, the present disclosure refers to a biomarker or combination of biomarkers of diagnostic, prognostic and monitoring of a flaviviral infection, wherein the biomarker comprises a complex formed by dimers of a flaviviral non-structural protein 1 (NS1) bound at the surface of plasma High Density Lipoprotein (HDL)particles as defined herein, and the combination of biomarkers further comprises a complex formed by a flaviviral NS1 and ApoB positive lipoprotein particles, and/or a complex formed by a flaviviral NS1 and ApoE positive lipoprotein particles. In yet another aspect, the present disclosure refers to the use of the presence or

level of the biomarker or combination of biomarkers disclosed herein in a blood sample obtained from a subject for the diagnostic, prognostic and monitoring of a flaviviral infection or associated disease. In a further aspect, the present disclosure refers to the use of an appropriate therapeutic agent in the manufacture of a medicament for treating a flaviviral infection in a subject in need thereof, wherein the subject had been diagnosed as having a flavivirus infection by detecting the presence or level of a complex formed by the flaviviral non-structural protein 1 (NS1) dimers and plasma High Density Lipoprotein (HDL)particles in a blood sample obtained from the subject according to the method disclosed herein, wherein the presence or level of the complex is indicative of a flaviviral infection in the subject. In one aspect, the present disclosure refers to a kit of diagnostic, prognostic and monitoring of a flaviviral infection in a blood sample obtained from a subject, comprising: a. an antibody specific for a flaviviral NS1; b. an antibody specific for ApoA1 and at least another antibody chosen from an antibody specific for ApoB and an antibody specific for ApoE; c. means for detecting the production of an immunoreaction product between said two antibodies a) and b) and the complex formed by the flaviviral NS1 and plasma lipoprotein particles.

DESCRIPTION OF THE INVENTION

The inventors found that Dengue virus NS1, secreted as an atypical lipoprotein particle by infected cells, binds with a strong affinity to serum high density lipoprotein particles (HDL), a major player of vascular homeostasis, inflammation and thrombosis, and to a lower extent to low density lipoproteins particles (LDL). The NS1-HDL complex, and not the NS1 protein alone, induces cytokine production in primary human macrophages suggesting that this complex represents a previously unreported active form of the viral protein. In parallel, the inventors developed three ELISA based on the NS1-ApoA1, NS1-ApoB and NS1-ApoE detection in DENV-infected patients to assess the concentration of complexes formed between NS1 and HDL, NS1 and LDL or between NS1 and a larger panel of lipoprotein particles (possibly HDL, IDL, LDL, VLDL and chylomicrons), respectively. The NS1-ApoA1 complex is detected in the majority of the acute phase blood samples and appears to be inversely correlated with the number of days of fever. In contrast, the concentration of the NS1-ApoE complex increases over time during the 12-days observation period. A biostatistical analysis of the different virological and clinical markers revealed that the

CLAIMS

1. An *in vitro* method of diagnostic, prognostic and monitoring of a flaviviral infection, comprising detecting the presence or level of a complex formed by dimers of a flaviviral non-structural protein 1 (NS1) bound at the surface of plasma High Density Lipoprotein (HDL) particles in a blood sample obtained from a subject, wherein presence or level of said complex in the blood sample is indicative of a flaviviral infection.

2. The in vitro method according to claim 1, comprising :
    a. Contacting the blood sample with an antibody specific for the flaviviral NS1 or an antibody specific for Apolipoprotein A1 (ApoA1) to form an immunoreaction product; and
    b. detecting the presence of the immunoreaction product.

3. The in vitro method according to claim 2, further comprising a step c. of quantitating the complex formed by the NS1 dimers and plasma HDL particles, after the detecting step in b.

4. The in vitro method according to claim 2 or 3, which comprises :
    a. Contacting the blood sample with an antibody specific for the flaviviral NS1 to form a first immunoreaction product;
    b. Contacting said first immunoreaction product with an antibody specific for ApoA1 to form a second immunoreaction product; and
    c. detecting the presence of the second immunoreaction product.

5. The in vitro method according to claim 4, further comprising a step d. of quantitating the complex formed by the NS1 dimers and plasma HDL particles, after the detecting step in c.

6. The in vitro method according to claim 3 or 5, wherein the quantity of the complex is determined by capture ELISA using the antibody specific for the flaviviral NS1 coated on a solid support and the antibody specific for ApoA1 as detection antibodies.

7. The in vitro method according to claim 4 or 5, wherein a third antibody raised against the antibody specific for ApoA1 and conjugated to a suitable label is used for detecting the presence of the second immunoreaction product.

8. The in vitro method according to any one of claims 1 to 7, wherein the plasma lipoprotein particles are Apolipoprotein A1 (ApoA1) positive lipoprotein particles.

9. The in vitro prognostic method according to any one of claims 1 to 8, which is for the prognostic of a severe form of a flaviviral infection in a subject infected with a flavivirus, wherein the more the level of said complex formed by dimers of the flaviviral non-structural protein 1 (NS1) bound at the surface of plasma High Density Lipoprotein (HDL) particles, the less the risk to develop a severe form of flaviviral infection.

10. The in vitro prognostic method according to claim 9, which is performed in a biological sample obtained during primary or acute infection.

11. The in vitro prognostic method according to any one of claim 9 or 10, wherein the plasma High Density Lipoprotein (HDL) particles are Apolipoprotein A1 (ApoA1) positive lipoprotein particles.

12. The in vitro monitoring method according to any one of claims 1 to 8, which is carried out on blood samples obtained from said subject at different times during the disease.

13. The in vitro diagnostic method according to any one of claims 1 to 8, which is repeatedly carried out on a blood sample obtained from said subject to further quantitate a complex of flaviviral NS1-ApoE positive lipoprotein particles, and/or a complex of flaviviral NS1-ApoB positive lipoprotein particles.

14. A biomarker or combination of biomarkers of diagnostic, prognostic and monitoring of a flaviviral infection, wherein the biomarker comprises a complex formed by dimers of a flaviviral non-structural protein 1 (NS1) bound at the surface of plasma High Density Lipoprotein (HDL) particles as defined in claim 1 or 8, and the combination of biomarkers further comprises a complex formed by a flaviviral NS1 and ApoB positive lipoprotein particles, and/or a complex formed by a flaviviral NS1 and ApoE positive lipoprotein particles.

15. Use of the presence or level of the biomarker or combination of biomarkers according to claim 14 in a blood sample obtained from a subject for the diagnostic, prognostic and monitoring of a flaviviral infection.

16. Use of an appropriate therapeutic agent in the manufacture of a medicament for treating a flaviviral infection in a subject in need thereof, wherein the subject had been diagnosed as having a flavivirus infection by detecting the presence or level of a complex formed by the flaviviral non-structural protein 1 (NS1) dimers and plasma High Density Lipoprotein (HDL) particles in a blood sample obtained from the subject according to the method of any one of claims 1 to 8 and 13, wherein the presence or level of the complex is indicative of a flaviviral infection in the subject.

17. A kit of diagnostic, prognostic and monitoring of a flaviviral infection in a blood sample obtained from a subject, comprising:
    a. An antibody specific for a flaviviral NS1;
    b. An antibody specific for ApoA1 and at least another antibody chosen from an antibody specific for ApoB and an antibody specific for ApoE;
    c. Means for detecting the production of an immunoreaction product between said two antibodies a) and b) and the complex formed by the flaviviral NS1 and plasma lipoprotein particles.

18. The kit according to claim17, the in vitro method according to any one of claims 1 to 13, the biomarker or use thereof according to claim 14 or 15 or the use according to claim 16, wherein the flaviviral NS1 is the NS1 from Dengue virus and/or the flaviviral infection is a Dengue virus infection.

19. The kit according to claim 17 or 18, the in vitro method according to any one of claims 1 to 13 and 18, the biomarker or use thereof according to any one of claims 14, 15 and 18, or the use according to claim 16 or 18, wherein the blood sample is plasma or serum.

20. The kit according to any one of claims 17 to 19, the in vitro method according to any one of claims 1 to 13, 18 and 19, the biomarker or use thereof according to any one of claims 14, 15, 18 and 19, or the use according to any one of claims 16, 18 and 19, wherein the subject is a human being.