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 for the diagnostic, prognostic or monitoring of a flaviviral infection or associated disease, comprising detecting the presence or level of a complex formed by a flaviviral non-structural protein 1 (NS1) and plasma lipoprotein particles in a biological sample obtained from a subject. In another aspect, the present disclosure refers to a biomarker for the diagnostic, prognostic or monitoring of a flaviviral infection or associated disease, comprising a complex formed by a flaviviral non-structural protein 1 (NS1) and plasma lipoprotein particles as defined herein. In yet another aspect, the present disclosure refers to the use of the presence or level of the biomarker disclosed herein in a biological sample obtained from a subject for the diagnostic, prognostic or monitoring of a flaviviral infection or associated disease. In a further aspect, the present disclosure refers to the use of an appropriate treatment in the manufacture of a medicament for treating a flaviviral infection or associated disease in a

subject in need thereof, wherein the individual 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) and plasma lipoprotein particles in a biological 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 to detect a complex formed by a flaviviral NS1 and plasma lipoprotein particles in a biological sample obtained from a subject, comprising: a. an antibody specific for the flaviviral NS1; b. an antibody specific for specific for plasma lipoproteins, preferably specific for HDL, and more preferably for ApoA1; and preferably further comprising an antibody specific for ApoB and/or ApoE; c. means for detecting the production of an immunoreaction product between said two antibodies and the complex formed by the flaviviral NS1 and plasma lipoprotein particles. In another aspect, the present disclosure refers to an in vitro method for quantitating a complex formed by the flaviviral non-structural protein 1 (NS1) and plasma lipoprotein particles in a biological sample obtained from a subject, comprising a. contacting the biological sample with an antibody specific for the flaviviral NS1 or an antibody specific for plasma lipoproteins to form an immunoreaction product; b. detecting the presence of the immunoreaction product, and c. quantitating the complex formed by NS1 and plasma lipoprotein particles ,according to the method disclosed herein.

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 for the diagnostic, prognostic or monitoring of a flaviviral infection or associated disease, comprising detecting the presence or level of a complex formed by a flaviviral non-structural protein 1 (NS1) and plasma lipoprotein particles in a biological sample obtained from a subject.

2. The in vitro method according to claim 1, comprising :
    a. Contacting the biological sample with an antibody specific for the flaviviral NS1 or an antibody specific for plasma lipoproteins to form an immunoreaction product;
    b. detecting the presence of the immunoreaction product, and
    c. quantitating the complex formed by NS1 and plasma lipoprotein particles.

3. The in vitro method according to claim 2, which comprises :
    a. Contacting the biological 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 plasma lipoproteins to form a second immunoreaction product;
    c. detecting the presence of the second immunoreaction product, and
    d. quantitating the complex formed by NS1 and plasma lipoprotein particles.

4. The in vitro method according to any one of claims 2 to 3, 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 plasma lipoproteins as detection antibodies.

5. The in vitro method according to any one of claims 2 to 4, wherein a third antibody raised against antibody specific for plasma lipoproteins and conjugated to a suitable label is used for detecting the presence of the second immunoreaction product.

6. The in vitro method according to any one of claims 1 to 5, wherein the plasma lipoprotein particles are High Density Lipoprotein (HDL) particles, preferably Apolipoprotein A1 (ApoA1) positive lipoprotein particles.

7. The in vitro method according to any one of claims 2 to 6, wherein the antibody specific for plasma lipoproteins is an antibody specific for Apolipoprotein A1 (ApoA1).

8. The in vitro method according to any one of claims 1 to 5, wherein the plasma lipoprotein particles are Low Density Lipoprotein (LDL) particles, preferably Apolipoprotein B positive lipoprotein particles.

9. The in vitro method according to any one of claims 2 to 5 and 8, wherein the antibody specific for plasma lipoproteins is an antibody specific for Apolipoprotein B (ApoB).

10. The in vitro method according to any one of claims 2 to 6 and 8, wherein the antibody specific for plasma lipoproteins is an antibody specific for Apolipoprotein E (ApoE)

11. The in vitro prognostic method according to any one of claims 1 to 10, 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 complex, the less the risk to develop severe form of flaviviral infection.

12. The in vitro prognostic method according to claim 11, which is performed in a biological sample obtained during primary or acute infection.

13. The in vitro prognostic method according to any one of claim 11 or 12, wherein the complex is formed by a flaviviral non-structural protein 1 (NS1) and plasma High Density Lipoprotein (HDL) particles, preferably Apolipoprotein A1 (ApoA1) positive lipoprotein particles.

14. The in vitro monitoring method according to any one of claims 1 to 10, which is carried out on biological samples obtained from said subject at different times during the disease.

15. The in vitro diagnostic method according to any one of claims 1 to 10, wherein the presence of complex formed by flaviviral NS1 and plasma lipoprotein particles in said sample is indicative of a flaviviral infection.

16. The in vitro diagnostic method according to claim 15, wherein complex formed by flaviviral NS1 and HDL particles are quantitated, preferably complex composed by flaviviral NS1 and ApoA1 positive lipoprotein particles.

17. The in vitro diagnostic method according to claim 15 or 16, which is repeatedly carried out on a biological sample obtained from said subject to quantitate complex of flaviviral NS1-HDL particles and/or complex of flaviviral NS1-LDL particles and/or complex of flaviviral NS1-other plasma lipoprotein particles, preferably complex of flaviviral NS1-Apo1 positive lipoprotein particles, complex of flaviviral NS1-ApoE positive lipoprotein particles, and/or complex of flaviviral NS1-ApoB positive lipoprotein particles.

18. A biomarker for the diagnostic, prognostic or monitoring of a flaviviral infection or associated disease, comprising a complex formed by a flaviviral non-structural protein 1 (NS1) and plasma lipoprotein particles as defined in any one of claims 1, 6 and 8.

19. Use of the presence or level of the biomarker according to claim 18 in a biological sample obtained from a subject for the diagnostic, prognostic or monitoring of a flaviviral infection or associated disease.

20. Use of an appropriate treatment in the manufacture of a medicament for ~~A method of~~ treating a flaviviral infection or associated disease in a subject in need thereof, ~~comprising:~~

~~- diagnosing a flavivirus infection in~~ wherein the individual 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) and plasma lipoprotein particles in a biological sample obtained from the subject according to the method of any one of claims 1 to 10 and 15-17, wherein the presence or level of the complex is indicative of a flaviviral infection in the subject~~; and~~

~~- administering an appropriate treatment if the individual is diagnosed with a flaviviral infection~~.

21. A kit to detect a complex formed by a flaviviral NS1 and plasma lipoprotein particles in a biological sample obtained from a subject, comprising:

    a. An antibody specific for the flaviviral NS1;

b. An antibody specific for specific for plasma lipoproteins, preferably specific for HDL, and more preferably for ApoA1; and preferably further comprising an antibody specific for ApoB and/or ApoE;

c. Means for detecting the production of an immunoreaction product between said two antibodies and the complex formed by the flaviviral NS1 and plasma lipoprotein particles.

22. An in vitro method for quantitating a complex formed by the flaviviral non-structural protein 1 (NS1) and plasma lipoprotein particles in a biological sample obtained from a subject, comprising:

a. Contacting the biological sample with an antibody specific for the flaviviral NS1 or an antibody specific for plasma lipoproteins to form an immunoreaction product;

b. detecting the presence of the immunoreaction product, and

c. quantitating the complex formed by NS1 and plasma lipoprotein particles ,according to the method of any one of claims 2 to 10.

23. The kit according to claim 21, the in vitro method according to any one of claims 1 to 17 and 22, the biomarker or use thereof according to claim 18 or 19 or the method of treatment according to claim 20, wherein the flaviviral NS1 is the NS1 from Dengue virus and/or the flaviviral infection is a Dengue virus infection.

24. The kit according to any one of claims 21 or 23, the in vitro method according to any one of claims 1 to 17 and 22, the biomarker or use thereof according to claim 18 or 19 or the method of treatment according to claim 20, wherein the biological sample is blood, plasma or serum.

25. The kit according to any one of claims 21 and 23 to 24, the in vitro method according to any one of claims 1 to 17 and 22, the biomarker or use thereof according to claim 18 or 19 or the method of treatment according to claim 20, wherein the subject is a human being.