CONFIDENTIAL

Report for the Penrose Inquiry - Specific Deaths - The late Mr. V.Tamburrini.

Query: whether or not SPPS (Stable Plasma Protein Solution) could have been the source of the HCV infection.

According to his medical history, the patient Mr. V.Tamburrini, was healthy until 1984 except for an appendectomy in 1968.
In September 1984, following an explosion and fire, he was admitted to hospital with severe skin burns (hands, forearms, face, back and thigh). As part of the treatment he received intravenously 6 units (each of 400 ml) of Stable Plasma Protein Solution (SPPS).

Manufacturing of SPPS by SNBTS
The administered units of SPPS were derived from the same lot (no.1194) which was manufactured in March 1983. The source material for this lot consisted of a pool of liquid plasma collected from blood donated by human subjects. According to the information provided by SNBTS (Dr.Perry), albumin was extracted from plasma through cold ethanol fractionation according to the method by Cohn et al (1950). In brief: this fractionation process involves the precipitation by ethanol of all undesired proteins with the desired protein(s) remaining in solution, or it can call for the precipitation of the desired protein(s) with all others left in solution. Ethanol at different concentrations is used to change protein solubility. With each change in ethanol concentration or pH different protein fractions are precipitated. Next, plasma fractions which are precipitated during ethanol fractionation are harvested by centrifugation or filtration. The removal of ethanol is accomplished by freeze-drying or ultra filtration. Finally the product - albumin - is pasteurized by heating for 10 hours at 60˚Celsius.

The Cohn fractionation method and its variations are used by many fractionators (both commercial and not-for-profit) around the world.

Like all human albumin, lot 1194 prepared by SNBTS for fractionation was manufactured against the monograph from the 1980 version of the British Pharmacopoeia (BP).

Heating procedure of SPPS used by SNBTS
After the product was dispensed in solution, caprylate was added to stabilize the protein, and bottles of 400 to 500 ml were placed in a spray chamber which sprayed water of 60˚Celsius over each of the bottles. The temperature was monitored using 6 temperature sensors placed in 6 bottles throughout the chamber. The batch manufacturing records show that the temperature was measured and recorded and confirm that the heat treatment process was controlled between 59.5˚Celsius and 60.5˚Celsius for a minimum of 10 hours.
According to BP the approved conditions of heat treatment were to heat in the final containers at 59.5˚Celsius to 60.5˚Celsius “so as to prevent the transmission

of hepatitis". The procedures for manufacturing and testing used by SNBTS complied fully with this requirement.

Hepatitis safety of albumin (including SPPS)

There are two ways to investigate the virus safety of plasma products such as albumin.

The first is to add (to spike) a known quantity of a (model) virus to the product and following the inactivation procedure determine the residual concentration of the virus still present in the product. As hepatitis C virus has not been isolated, a model virus BDV (bovine diarrhoea virus) is commonly used for this validation of the inactivation of hepatitis C. The process of cold ethanol fractionation significantly reduces the concentration of viruses in plasma fractions. The albumin pasteurisation process is also known to remove the risk of transmission of infectious agents by denaturation of viral proteins and nucleic acids, thereby inactivating viruses. (Ref. *Erstad, B.L. Viral infectivity of albumin and plasma protein fraction. Pharmacotherapy 16, 996-1001, 1991; Yei, S. Ya, M.W. Tankersley, D.L. Partitioning of hepatitis C during Cohn-Oncley fractionation of plasma. Transfusion 1992; 32:824 – 828; Schleibaum, H. Nubling, M.Wilkommen, H. and Lower, J. Prevalence of hepatitis C virus in plasma pools and effectiveness of cold ethanol fractionation. Clin. Ther. 1996: 18; 59 -70).* These studies have shown that heating of albumin for 10 minutes at 60˚Celsius results in a virus reduction (log 10) of > 16.3 of BDV (as compared to > 17.8 of HIV and 16.4 of pseudorabies (model virus for hepatitis B). These levels of virus inactivation and reduction are recognised as providing a very high margin of safety for products of this type. Please note that the pasteurization of albumin takes 10 hours at 60˚Celsius, which 60 times longer than is needed to inactivate hepatitis viruses.

As SPPS is essentially composed of albumin it is allowed to apply the evidence of safety as published for albumin to SPPS.

The second way is to investigate if patients treated with albumin or SPPS have developed hepatitis C. There has never been a report of hepatitis C transmission through albumin infusion. In 1976 one hepatitis B outbreak was reported in relation to PPF (=SPPS) infusion (*Ref.Patisson, C.B. et al: An outbreak of hepatitis B associated with transfusion of plasma protein fraction. Am.J. Epidemiology 1976; 103: 399 – 407*). However it is believed that this isolated incident probably resulted from a lack of uniform heating of the bulk product during pasteurisation. This incident has led the FDA in the United States to require that the heating step (pasteurization) takes place after the product is placed in individual containers (Code of Federal Regulations, Title 21, CFR 640). Since that time there have been no other reports of hepatitis B transmission through albumin products.

The hepatitis safety of albumin is addressed in reports of several healthcare authorities.

As mentioned before, the British Pharmacopoeia requests heating in the final containers at 59.5˚Celsius to 60.5˚Celsius "....to inactivate hepatitis viruses".

The Expert Committee on Biological Standardization of the World Health Organisation (WHO) is an internationally recognized independent body that regularly reviews issues like the safety of blood products and the various methods used to safeguard the quality of such products. In 2001 this Committee has adopted an extensive report called "Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma

products" (WHO Technical Reports Series no. 924, annex 4). In paragraph 4.1.1, where the pasteurization of albumin is described, it is stated that: "Safety with respect to hepatitis viruses and HIV (human immunodeficiency virus) has been demonstrated for decades, with few exceptions (*ref.11 Pattison C.B. et al An outbreak of type B hepatitis associated with transfusion of plasma protein fraction. Am.J. Epidemiology 1976 103 399 - 407)*". (The latter exception has been described above).
Data in this WHO-report show that infectious virus can no longer be detected after 10 minutes of heating at 60 °Celsius, which is much shorter than the time used for the pasteurisation process of albumin.

Conclusion
The batch of SPPS administered to Mr Tamburrini was manufactured using methods which were at the time (and still are) widely recognised as being capable of eliminating any risk of virus transmission. The records of batch number 1194 indicate that its manufacture, and in particular its pasteurisation, was carried out according to recognised industry and pharmacopoeial standards.
The answer to the query therefore is that transmission of hepatitis C by SPPS is most unlikely as the Cohn fractionation method used by SNBTS has been shown to significantly reduce the concentration of viruses in plasma fractions and the method of pasteurisation for SPPS has been demonstrated to cause complete virus inactivation (including hepatitis C). In addition, albumin and SPPS have been used worldwide for several decades and transmission of hepatitis C has not been reported in the literature.

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