The Penrose Inquiry

C2 – Surrogate testing of Blood for Non-A, non-B Hepatitis

Supplementary Questions – Response of Dr John Gillon

1) In respect of the 1984(sic) paper by Crawford et al (PEN.002.0582), what was meant by "ALT levels above the upper limit of normal" (i.e. what was the upper limit of normal, expressed as IU/L)?)

Routine testing of blood donors for antibodies to HCV began in SNBTS on 1st September 1991. Donors confirmed to be positive were counselled according to guidelines approved by SNBTS Directors, and this included taking a blood sample for liver function tests, including ALT, carried out by the respective Departments of Clinical Chemistry in the five SNBTS Regions. There were no plans to carry out research prospectively on these donors, but at a later date it was decided that the information obtained through counselling and extended testing of these donors merited analysis and preparation for publication.

The data presented in Crawford et al, 1994 (PEN.002.0582) were collated originally by Dr Robert Crawford from reports provided by nominated consultants in each of the 5 SNBTS Regions. As stated in the paper, ALT tests were carried out in the local Departments of Clinical Chemistry on blood samples taken at the time of counselling donors with confirmed positive tests for hepatitis C. Different laboratories used different test methods, and had different upper limits of normal. The standard method for determining normal levels is to test a sufficiently large population of "normal subjects" to allow valid statistical calculations to be made. The upper limit of normal is then taken as 2 standard deviations (SD) above the logarithmic mean of all results. This then defines the upper 2.5% of that population. Individual laboratories define their own levels of normality using a local population sample, for ALT as for other tests.

Thus, the results obtained on the HCV positive donors were defined in relation to their local populations, not to the Scottish blood donor population (for whom no such data were in existence). I have no record of the levels that were in use in the regional laboratories at that time, but because of the variations in

test methodology and local demographics they are likely to have been different from one another, but not markedly so.

A discussion of the implications and relevance of ALT cut-off levels is to be found in my response to Question 5 in "Other queries", below.

2) Whether or not there is any information available as to how many anti-HCV negative Scottish blood donors had ALT levels above the upper limit of normal?

To the best of my knowledge no representative sample of known HCV negative Scottish blood donors has ever been screened for ALT levels. However, two cohorts of Edinburgh donors were screened in the period prior to screening tests for HCV becoming available, with the aim of ascertaining the potential impact of ALT testing, should it be introduced in Scotland.

The Inquiry is already aware of the data reported in a letter to the Lancet in 1987 and later published in full in Vox Sanguinis in 1988 (Gillon et al, Vox Sang 1988;54:148-153: **SNB.008.3536**). In this study we measured ALT levels in 1742 regular blood donors using the standard method in the Department of Clinical Chemistry, Royal Infirmary of Edinburgh. We did not calculate an upper level of normal using these results, but instead chose to use 45 units/L as the upper limit of normal in order to facilitate comparison with published data from the USA. The result of this was that 2.4% of donors were found to have raised ALT levels by this definition, indicating that the chosen level was, in fact, very close to 2SD above the log mean.

We also reported in the same paper a survey of the records of 708 plasmapheresis donors, who had ALT levels measured by the same routine methods prior to their first plasma donation and at 6 month intervals thereafter. 3.7% of these highly selected "pedigree" donors had raised ALT on the first sample, and 6% of those with initially normal levels had an elevated level at some point thereafter. This finding led us to study plasma donors in more detail, as reported in the following paper:

Prowse C, Picken M, Gillon J. Prevalence and Consistency of ALT elevation in Plasmapheresis Donors: Implications for the Assessment of Blood Product Infectivity. ***Vox Sang 1993; 65: 204-208.***

This study was carried out before testing for anti-HCV became available, so no correlation with demonstrated evidence of HCV infection was possible. However, we showed that by measuring ALT levels at every attendance of 431 donors (plasma donors typically donate monthly) over a period of 18 months, 23% had an elevated ALT at some point, and 11.1% of donations exhibited ALT levels >40 IU/L, the upper limit of normal for the laboratory at that time. Analysing the data by sex showed that 14.8% of donations by men and 3.6% of donations by women had levels >40IU/L. 58% of men had at least one ALT over 40IU/L at some time, compared with 22% of women. This confirmed previous reports of a difference in ALT level in men and women. We analysed the initial samples of all donors according to sex (279 males vs 152 females), and using 2SD above the log mean for each group separately as the upper limit (60.8 IU/L for men, 40.2IU/L for women), 2.7% of all donors had an elevated ALT initially (males 2.2%, females 3.6%).

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Other queries

(1) Should a large scale prospective study, as originally proposed by Dr McClelland in 1981 (i.e. along the lines of the US TTV and NIH studies and including the follow-up of recipients), have been carried out in the UK in the early 1980s (or at some point thereafter) with the following aims;

(a) to assess the prevalence of post transfusion NANBH in the UK,

(b) to evaluate surrogate markers for the disease,

(c) to investigate the natural progression and seriousness of the disease, and,

(d) to produce a library of "known" infected sera with which to evaluate any future assays which became available?

I shall make a few general comments in respect of this query, and will give a more detailed response to the question of the effectiveness and potential outcomes of surrogate testing in my response to query (5) below.

With hindsight it easy to say that such a study would have been desirable, though it would have been incredibly prescient in 1981, as the significance of NANBH was only then becoming apparent, and indeed there was at the time and for many years thereafter a body of opinion which disputed the seriousness of chronic NANBH. A programme of research into transfusion transmitted hepatitis had been established in WBTS for many years, and in SEBTS basic research to try to detect markers of putative infectious agents was established by the early 1980s, in collaboration with research teams in the US and elsewhere.

Information on the prevalence of the disease and outcome for patients would have been highly desirable, the former in order to estimate the possible effectiveness of interventions such as surrogate testing (which was first suggested by the TTV study published in 1981), and the latter to define the severity of the potential sequelae of PTNANBH. The difficulties involved in carrying out a prospective study should not, however, be underestimated. The American studies (TTV and NIH) were carried out in the context of very high PTNANBH rates, which meant that a relatively large number of affected patients could be identified relatively quickly. Though there were no reliable data for the incidence of PTNANBH in the UK, there was little to suggest that the disease was occurring at the rate seen in the USA. A multicentre study would almost certainly have been necessary, making great demands in terms of resources and manpower. Such studies are therefore very expensive, and do not provide quick answers. In the context of a disease about which very little was known and for which no specific diagnostic test was available, I think it is unsurprising that such a study was not pursued in 1981 or shortly thereafter, particularly once it was acknowledged that a definitive answer to the question of the efficacy of surrogate tests could not be obtained by such studies, but only by a prospective randomised trial in which sufficient numbers of patients were randomised to receive either ALT screened or unscreened blood. No such trial was ever carried out on a scale big enough to provide definitive answers.

(2) If such a study had been carried out, to what extent is it likely to have met the objectives set out in (1) above? To what extent would such a study have provided more information upon which to base a decision on whether surrogate testing should have been introduced?

The success of such a study would have depended critically on a variety of factors, but it is likely that an evaluation of the prevalence of PTNANBH in the UK could have been obtained (with the important caveat that diagnosis in the absence of a specific test would be likely to be very imprecise), and would have been useful in deciding whether or not to introduce surrogate testing.

Investigating the natural history of the disease, however, would not have been within the remit or capabilities of the transfusion services.

In order to be of value, a bank of sera for future use would need to have been substantial in the numbers of affected patients, controls, and their respective donors, as would have been necessary to establish an accurate estimate of prevalence. In a putatively low prevalence population such as was thought to be the case in the UK, an enormous number of patients would have had to be followed up.

(3) I have no comment on the conclusions drawn by Drs Dow and Follett.

(4) I have no comment to make on this issue.

(5) If surrogate testing of blood donors (i.e. testing for elevated ALT and/or anti-HBc) had been introduced in Scotland:

(a) what percentage of donors are likely to have been deferred,

In the study carried out in Edinburgh and published in 1988 (SNB.008.3536) 2.4% of donors had ALT >45 units/L, and 2.0% were positive for anti-HBc, with no overlap between the two groups. This suggests a minimum donor loss of 4.4% if both tests were implemented. The donor loss due to anti-HBc requires no further elaboration, since this is a specific marker for past exposure to HBV, and is thus in a different category from ALT in that it is readily understood that this could function as a valid surrogate marker for past exposure to other parenterally transmissible viruses. The finding of an association between anti-HBc in donors and recipient NANBH was a surprise outcome of the TTV study, and the most surprising aspect was the lack of overlap between donors with raised ALT and those with anti-HBc. This led the authors of the NIH study to speculate as follows:

"This dichotomy is disturbing, suggesting either that the tests are not really detecting carriers of non-A, non-B hepatitis and that their apparent association with non-A, non-B hepatitis is a statistical artefact, or that they are detecting two different carrier populations perhaps harbouring different agents for non-A, non-B hepatitis." (Alter HJ and Holland PV, Ann Int Medicine 1984; 101:859-861).

The main difficulty with anti-HBc as a surrogate screening test was the technical limitations of the tests then available and that lack of suitable confirmatory tests for those donors who did not have other markers of HBV infection. The American experience when surrogate testing was implemented in 1987 was that anti-HBc was indeed problematic, and for these reasons it has never been implemented in the UK, in spite of the apparent logic behind it.

The case of ALT is entirely different, in that this enzyme is a normal constituent of blood and is therefore present universally. The blood level varies with body weight, increasing with increasing weight, and levels above the standard "upper limit of normal" of 2SD above the log mean are associated with obesity. Though this association was recognised in the 1980s, it was poorly understood, and hepatologists were at that time puzzled by the increasing numbers of patients they were seeing with abnormal liver function tests discovered coincidentally. Later research revealed that the abnormalities of liver function were due to fatty infiltration of the liver. The association was not only with obesity, but also with insulin resistance and type II diabetes, and this constellation was named "the metabolic syndrome", with the liver component designated "non-alcoholic steatohepatitis (NASH)".

Higher mean levels of ALT are found in men as compared with women, as we found in our study of plasma donors (Prowse et al 1993), and it is thought that this is largely due to differences in body weight. The segment of the population with the highest mean levels is in males aged 30 – 40. Higher levels are also found in association with excessive alcohol consumption, and are associated with muscle damage. e.g. myocardial infarction, but more commonly after hard exercise.

In most studies of HCV positive blood donors there is found to be a preponderance of males, typically in the age range 30-40. There is therefore a

coincidental association between higher ALT levels and the donors most likely to have been exposed to HCV. It is therefore likely that ALT is to some extent an epiphenomenon in statistical or epidemiological terms, as Alter and Holland suggested. This may well have been an example of the "fallacy of the transposed conditional", first proposed by Falk in 1986 (Falk R. Conditional probabilities: insights and difficulties. In Davidson R and Swift J (eds); Proc 2nd International Conference on Teaching Statistics, pp292-297. International Statistics Institute, Victoria, Canada). In essence, this can be rendered as follows: if a patient develops PTNANBH, there is a strong possibility of having received blood with a high ALT (because of the coincidental segregation of the carriage of NANBH and raised ALT), whereas a raised ALT in a donor says little about the risk of NANBH in the recipient.

There is also, of course, a more specific sense in which ALT can be regarded as a "surrogate" for HCV, as opposed to being a mere statistical artefact, inasmuch as it is often raised in the presence of liver damage, though again this is not specific to any particular cause of liver damage. There is therefore *prima facie* reason to think that ALT testing might prevent some PTNANBH.

Even when a raised ALT is found in the presence of HCV, and can therefore reasonably be attributed to the effect of the virus on the liver, the levels are known to fluctuate within an individual. The same is true of "normal" donors, as shown in our study of plasma donors (it is very unlikely that any of those donors were HCV positive), so different results would be obtained from the same population at different times. Thus, repeated testing of the same population of donors would give a much higher "hit rate" for raised levels than a one-off snapshot, with obvious implications for the potential impact on the blood supply.

The crucial issue here is the choice of cut-off level, and this is indeed the crux of the problem posed by ALT testing. There is, in fact, no true "upper level of normal" that accurately differentiates between those with disease and those without disease. This tension between true positive and false positive, which can be characterised as sensitivity vs specificity, is a characteristic of even the most accurate and specific tests, but is magnified in the case of a normal biological variable as opposed to a specific marker of infection. The higher the cut-off, the fewer true positives will be detected, resulting from a loss of

sensitivity (defined as the ability of the test to identify true positives), but a smaller proportion of those identified by the test will be false positives, indicating greater specificity. Set the cut-off at a lower level and the position is reversed, with more of the true positives being identified but at the expense of larger numbers of false positives, with all that implies for the individual donors and for the impact on the blood supply. The choice of cut-off level is thus entirely arbitrary, and in the present context boils down to striking a balance between the need to identify as many of the HCV infected donors as possible and the desire to minimise the impact on non-infected donors, and on the blood supply.

It is thus impossible to state what percentage of donors would have been deferred. I am not aware of the cut-off level having been the subject of debate within SNBTS or at UK level. In the USA, a compromise position was adopted, whereby donors with modestly elevated levels were not informed, but the donation discarded, while those with higher levels, and those with repeated modest elevations, were informed and deferred.

(b) could a sufficient blood supply have been maintained, and

This, too, being hypothetical and subject to various assumptions, not least the choice of cut-off level as described above, is impossible to answer with any degree of certainty. There was difficulty in maintaining donor attendances in the second half of the 1980s, for reasons that were not fully understood. The situation became serious to the extent that a substantial injection of resources was necessary around 1990, with most of the money and effort going into a television advertising campaign which reversed the decline in donor numbers. Whether it would have been possible to weather a loss of donations of the order of at least 4-5% and so maintain self sufficiency with or without such funding is doubtful, but this is speculative in the extreme.

(c) to what extent are cases of post-transfusion hepatitis C likely to have been prevented (having regard, for example, to the finding in the first six months of HCV screening that the prevalence in Scottish blood donors was 0.088%, and that elevated ALT levels were found in 59% of HCV positive donors)?

Estimates of the number of cases of PTHCV occurring annually in Scotland during the period prior to the introduction of a test for anti-HCV will be presented to the Inquiry by Prof David Goldberg. The starting point for these estimates was the prevalence found in SNBTS donors between 1 September and 31 December 1991, i.e. 0.09%, the assumption being that very few donors would have been able to donate more than once during that period (the obvious exception being plasma donors who mostly donate monthly; their numbers, however, were very small). Backwards chronological extrapolation, incorporating a number of assumptions including the change in prevalence in the population with time, will permit the most accurate estimates to date of the number of patients exposed to potentially infectious donations.

It is impossible to state with certainty what proportion of these potential (and actual) transmissions might have been prevented by ALT testing. Though the percentage of donors in the HCV positive cohort with raised ALT is impressive (59%), it cannot be assumed that a similar proportion of PTHCV would have been prevented by ALT screening. Much would have depended on the choice of cut-off, but it should be borne in mind that in a low prevalence population like that in Scottish blood donors, the ratio of false to true positives would be very high, at all but a very high cut-off value.

In concluding, I would wish to emphasise that neither ALT nor anti-HBc testing has ever been shown, in a randomised trial of sufficient power, to prevent PTHCV. Alter and colleagues, based on their experience in an environment where PTNANBH rates were around 10%, estimated that ALT testing might prevent around 30% of transmissions, yet in their prospective study after initiating routine ALT testing in 1981 (without a control group; this was not a controlled trial), they found no reduction in NANBH incidence in transfused patients (Klein HG, Transfusion 1990; 30: 363-367). In a low prevalence population such as Scottish blood donors, ALT testing would be unlikely to exceed the efficacy found in the USA, and so to suggest that a more advantageous outcome might have been obtained on this side of the Atlantic would be pure speculation.

Dr J Gillon, October 2011

Statement of truth

I believe that the facts stated in this witness statement are true

Signed: ……

Dated: ……