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Post-transfusion hepatitis: impact of non-A, non-B hepatitis surrogate tests

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Summary

Canada has not introduced the non-A, non-B (NANB) surrogate marker tests (antibodies to hepatitis B core antigen and alanine aminotransferase) to screen donated blood. We evaluated the effect of NANB surrogate markers in reducing post-transfusion hepatitis in a prospective randomised intervention study.

From 1988 to 1992, 4588 subjects were enrolled into two study groups that received allogeneic blood from which units positive for NANB surrogate markers were either withheld (n=2311) or not withheld (n=2277). We also assessed a simultaneous non-randomised cohort (n=650) of subjects who received only syngeneic blood. All subjects were followed up for 6 months and assessed for the presence of post-transfusion hepatitis due to hepatitis A, B, C, non ABC, Epstein-Barr virus (EBV) and cytomegalovirus (CMV). Withholding of blood containing NANB surrogate positive units reduced the overall post-transfusion hepatitis rate by 40% (p=0·065) and the hepatitis C rate by 70% (p=0·05). Most of the benefit of NANB surrogate testing was due to reduced frequency of hepatitis C virus after transfusion before all donor blood was screened for anti-HCV. During this time the overall post-transfusion hepatitis rate per 1000 transfusion recipients was 20·2 in the no-withhold group compared with 5·0 in the withhold group (p=0·05), and the HCV hepatitis rate was 12·6 and 0 respectively (p=0·06). After the introduction of HCV screening, the overall post-transfusion hepatitis rates were 8·6 and 6·8 per 1000 (p=0·55) respectively.

Our study indicates that screening of blood donors with the NANB surrogate markers was of value in reducing HCV infection before HCV screening began, but subsequently the value of screening cannot be clearly established.

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Introduction

A prospective study of post-transfusion hepatitis in Canada in 1984–85 showed an overall post-transfusion hepatitis frequency of 92 per 1000 allogeneic blood recipients, with a post-transfusion frequency of hepatitis C (HCV) of 31 per 1000 recipients.[1,2] Since 1985, many measures were introduced by blood-collection agencies world wide to try to improve the safety of the blood supply. These included the introduction of screening for HIV-1, human T-cell lymphotropic virus (HTLV) type I, HBsAg, and direct questioning of blood donors about relevant medical information and lifestyle.[3,4] In 1986, agencies in the USA introduced two tests to screen blood donors, to reduce the frequency of non-A, non-B (NANB) post-transfusion hepatitis. Thus, donors with an increased concentration of the hepatic enzyme alanine aminotransferase (ALT) or the presence of antibodies to the hepatitis B core antigen (anti-HBc) were excluded from donating blood. These two NANB surrogate markers were used because of results from two prospective studies of allogeneic blood product recipients in the 1970s.[5,6] This decision was made without the benefit of data from prospective intervention studies showing efficacy.

Because of the lack of such evidence, the Canadian Red Cross Society and some blood transfusion services in western Europe did not screen blood donors for NANB surrogate markers. We thought a randomised double-blind trial was needed in Canada to assess the frequency of post-transfusion hepatitis and so see whether the withholding of donor blood positive for the NANB surrogate markers would reduce the frequency of post-transfusion hepatitis.

While our study was in progress the genome of HCV was elucidated.[7] Testing blood donors for antibodies to HCV was introduced in Canada in May, 1990. Subjects were involved in our study before and after the introduction of anti-HCV testing.

Patients and methods

Patients

Participating institutions included 3 Canadian Red Cross Society Blood Centres (Hamilton, Toronto, and Winnipeg), and 13 university-affiliated hospitals (6 with McMaster University, 5 with the University of Toronto, and 2 with the University of Manitoba). Consecutive adult patients who required transfusion (red blood cells and/or plasma) and were admitted to participating hospitals, were screened for eligibility by trained research personnel before transfusion. We obtained written informed consent from each subject before study entry. Specifically, we told potential subjects that previous results suggested that NANB surrogate tests could predict whether blood donors carry viruses that could cause post-transfusion hepatitis. Exclusion criteria were: the presence of red cell alloantigens that would have made the provision of blood products difficult; chronic liver disease; known alcohol abuse;

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blood product transfusion in the past 6 months; presence of malignant disease with metastases; a medical condition with a life expectancy of less than 9 months; and a pre-surgery concentration of ALT higher than 1·5 times the upper limit of normal. Subjects who did not need a transfusion were not included in the final study population.

Eligible subjects were blindly randomised to one of two allogeneic blood recipient groups between 1988 and 1992. One group (n=2311) received only allogeneic blood products that did not contain NANB surrogate markers (withhold group). The other group (n=2277) received allogeneic blood products that had only routine Canadian transfusion-transmissible disease marker screening (non-withhold group), and may have been given blood products that were positive for the NANB surrogate markers.

We took a pre-transfusion serum sample from each subject to determine alanine aminotransferase concentration. Baseline data for each subject included relevant demographic data; the type and number of blood products transfused; the type of surgery; medications; and perioperative hypotensive episodes. Our protocol specified that all subjects would be followed up for 6 months after blood transfusion with visits at 3, 6, 8, 12, and 24 weeks. At each visit, we took a blood sample for alanine aminotransferase measurement and asked the patient to fill in a short questionnaire. We stored each sample taken for alanine aminotransferase in aliquots at −70°C. All blood products we used on our subjects were tested and were negative for: VDRL, HBsAg, HIV-1 antibodies, HTLV-2 antibodies, and from May 1, 1990, the first generation anti-HCV test.

To determine the background frequency of post-transfusion hepatitis, we also studied a simultaneous cohort of 650 non-randomised control subjects having surgical procedures but receiving only autologous blood. This group had the same requirements for informed consent, eligibility, and follow-up procedures. We thus had three study groups.

Laboratory methods

All blood samples were tested in a central laboratory at the liver study unit at Mount Sinai Hospital. We measured alanine aminotransferase concentrations for each study subject on unfrozen samples, within 24 hours of collection, by an automatic kinetic assay (VP batch analyzer and reagents, Abbott Laboratories, North Chicago, IL, USA). Recipient blood samples, taken at 24 weeks after transfusion, were also tested for anti-HBc with a short-incubation ELISA (Corzyme, Abbott Laboratories). If the 24-week sample was positive and the pre-transfusion sample negative for anti-HBc, all available serum samples were tested for HBsAg, IgM anti-HBc, anti-HBs antibodies, HBeAg, HBe antibodies and HBV DNA by dot hybridisation.[9] These serum samples also had PCR analysis with primers from the surface and core genes of the HBV genomes.[8]

Any sera that had alanine aminotransferase above 2·5 times the upper limit of normal (ie, 40 IU/L), at any point after transfusion, were then screened for serological markers of hepatitis A, B, C, EBV and CMV. Initially, we used a first generation HCV ELISA (Ortho Diagnostics, Rariton, NJ, USA) together with recombinant immunoblot (RIBA-I, Chiron, CA, USA). Subsequently, we retested each serum sample with second-generation HCV ELISA together with a second-generation confirmatory assay (RIBA-II, Ortho Diagnostics). We also tested the 24-week serum samples of all study subjects with a second-generation HCV ELISA. In addition, the pre-transfusion serum samples from all study subjects who were confirmed positive for HCV antibodies at 24 weeks, were again screened for anti-HCV and for HCV RNA, with nested primers for the 5′ untranslated region of HCV, followed by Southern blotting.[7,9] Tests for CMV and EBV were performed by the Virology Department of The Ontario Public Health Laboratory, Toronto. CMV serology was tested by both complement fixation and ELISA (Behring Diagnostics, Montreal, Canada); EBV by immunofluorescence and ELISA (Ortho Diagnostics, Ranton, NY, USA).

	No-withhold (n=2277)	Withhold (n=2311)
Study centre		
Hamilton	35·3	34·2
Toronto	49·2	49·1
Winnipeg	15·5	16·7
Characteristics		
Sex (% male)	54·3	53·3
Mean age (yr)	63·1	62·5
Race (% caucasian)	94·2	93·8
Occupation		
Retired	60·0	59·6
White collar or professional	16·7	15·4
Education (% high school or more)	51·9	48·6
Married	69·5	67·8
Current smoker	12·5	14·0
Previous transfusions	51·7	54·2
Indication for transfusions		
Blood loss	90·0	90·6
Surgery	92·8	93·2
Cardiac	52·5	53·2
Orthopaedic	34·0	33·6
Number of transfusions		
1	14·2	13·9
2–4	61·5	61·9
5–9	16·6	17·2
>10	7·7	7·0
Blood products received		
RBC only	83·5	83·6
RBC and plasma or plasma alone	10·8	11·7
RBC and other or other alone	5·7	4·6
Non study or misallocated blood received	15·4	14·7
Follow-up adequate	88·3	87·6
Drop-out, no tests after 5 weeks	8·0	7·9

RBC: red blood cells. Data are %.

Table 1: **Characteristics of the subjects in the two randomised study groups**

Classification of post-transfusion hepatitis events

Subjects were classified as having post-transfusion hepatitis when: the serum alanine aminotransferase concentrations were increased to at least 2·5 times the upper limit of normal; the alanine aminotransferase of a second serum sample was increased to at least twice the upper limit of normal within 7–10 days of the initial increase; and other potential causes of abnormal liver function were excluded. The final post-transfusion hepatitis diagnosis of each subject was made by an events committee, consisting of an infectious disease specialist and two hepatologists. The committee was not involved in any other part of the study and was unaware of the transfusion group when reviewing data. We based the aetiological diagnosis of post-transfusion hepatitis caused by either HBV or HCV on serological variables and HBV DNA needed to be detected within 12 weeks of transfusion. Post-transfusion HCV was diagnosed when HCV was detected by a second-generation ELISA and confirmed with a recombinant immunoblot assay and/or presence of HCV RNA by PCR.[10] Subjects whose pre-transfusion sample was HCV positive were not regarded in the

	Surrogate test group		Comparison of no-withhold with the withhold group	
	No-withhold (n=2277)	Withhold (n=2311)	p value*	Risk benefit
Number of recipients	2277	2311		
Overall post-transfusion hepatitis cases	24	15	0·065	40%
HCV hepatitis cases	10	3	0·048	70%
Non-ABC hepatitis cases	14	12	0·65	16%

*Mantel-Haenszel test statistic stratified by time (pre-HCV screening versus post-HCV screening) and centre. The test is one-sided for the primary outcome, overall post-transfusion hepatitis, but two-sided for the secondary outcomes, HCV hepatitis and non-A, non-B, non-C (non-ABC) hepatitis.

Table 2: **Observed post-transfusion hepatitis events in the two randomised study groups**

	Number of recipients	Number of events	Overall post-transfusion hepatitis (95% CI)	Hepatitis* (95% CI)	
				HCV-PTH	non-ABC-PTH
Pre-HCV screening					
No-withhold	397	8	20·2 (8·7–39·3)*	12·6 (4·1–29·1)	7·6 (1·6–21·9)
Withhold	402	2	5·0 (0·6–17·9)	0·0 (0·0–7·4)	5·0 (0·6–17·9)
Post-HCV screening					
No-withhold	1880	16	8·6 (5·9–13·8)	2·7 (0·9–6·2)	5·9 (2·9–10·4)
Withhold	1909	13	6·8 (3·6–11·6)	1·6 (0·3–4·6)	5·2 (2·5–9·6)

Table 3: **Post-transfusion hepatitis rates per 1000 recipients of allogeneic blood products in the pre-HCV and post-HCV screening periods**

final analysis as having post-transfusion hepatitis events. All study subjects who had an alanine aminotransferase concentration above 2·5 times normal and, 7 to 15 days later, higher than 2 times the upper limit of normal, and were negative for HBV and HCV, were designated as having non ABC post-transfusion hepatitis. These subjects must not have had any other causes of alanine aminotransferase increase.

Protocol adherence

We stopped recruiting patients at the end of January, 1992, and the database was closed at the end of January, 1993. 4597 subjects were included in the final cohort. We excluded 9 subjects from the intent-to-treat analysis because we did not have transfusion data (n=5) or because we could not determine the group allocation (n=4), but there were no post-transfusion events in these 9 subjects. Some participants had blood products that were inconsistent with their randomised allocation (table 1)—blood intended for the other group, or blood that had not been pre-screened for the surrogate markers (non-study blood). This occurred because there were, on occasion, shortages of blood units for the assigned group of the appropriate blood type, and/or cross-matching difficulties. Follow up was deemed to be adequate when there were at least 3 blood samples, or which at least one was taken before 12 weeks post transfusion and at least one was taken after 12 weeks. 88% of study subjects met these criteria. Sensitivity analyses to assess the effects of deviations from group allocation, incomplete follow-up, and drop-outs, did not significantly change the study's findings.

Design specifications and statistical methods

Our protocol specified a total sample size of 3200, with one interim analysis when the first 1600 subjects had completed follow-up. This sample size was expected to provide power to detect a 40% benefit with an overall one-sided type-1 error rate of 5%, assuming a no-withhold post-transfusion hepatitis rate of 5%, a withhold rate of 3%, and a 20% loss-to-follow-up rate. To account for the introduction of HCV screening and an overall post-transfusion hepatitis rate that was lower than expected, the sample size requirements were recalculated before we did the interim analysis. A post-transfusion hepatitis rate of 3% was assumed in the time before HCV screening and 2% in the period after HCV screening. A detectable benefit for surrogate testing was 50% in both periods.[11] The target sample size was increased to 4700, with allowance for two interim analyses;[12] however, no interim analyses were done during the study.

Rates per 1000 subjects of overall post-transfusion hepatitis, HCV hepatitis and non-A, non-B, non-C hepatitis were calculated with exact 95% CIs. Our primary analysis compared the frequency of post-transfusion hepatitis in the withhold group with that in the non-withhold group by intention-to-treat Mantel-Haenszel analysis stratified by centre and by time. Homogeneity of odds ratios among the strata was assessed by the Breslow-Day test.

We used logistic regression to assess the effect of surrogate testing for each time-period separately and for the secondary outcomes, HCV hepatitis and non-A, non-B, non-C hepatitis with covariates for group and time, and with and without stratification by centre.[13] We used two-sided tests because these analyses had not been specified in the original design. We calculated the risk benefit and corresponding 95% CI from the logistic regression odds ratio estimates of the relative risk for the withhold group. Because of the small numbers of events for the secondary outcomes by time-periods, we also did exact logistic regression analyses. To increase the low power of the standard logistic regression test for differential surrogate testing effects between the two time periods we pooled the data from the withhold groups for blood donated before and after HCV screening.

We estimated the relative risks associated with transfusion of a marker-positive unit in each period for each of the outcomes with logistic regression disregard odds ratios. Indicator covariates for the type of donor units received (any units that were positive for HCV antibodies or any units with alanine aminotransferase units above 40) were used to investigate the association with each marker on its own and in combination. Tests for differential effects by time were done by including interactions between time-period and unit type. The associations of post-transfusion hepatitis with the total number of units transfused and with the numbers of negative and positive units transfused were investigated by logistic regression. We used Poisson regression to estimate per unit rates and CIs with an offset for the numbers of units transfused.

Results

Frequency of post-transfusion hepatitis

Table 1 shows the demographic and other characteristics of the study subjects in the two allogeneic transfusion groups. The participants in the study groups were generally similar in sociodemographic, and transfusion characteristics. About 15% of subjects received non-study or misallocated blood. Exclusion of these cases from the analysis did not change the study findings. The non-randomised cohort of recipients of syngeneic blood differed in several respects from those in the non-withhold and the withhold groups: they were younger and were more likely to be female, white collar/professional workers, and to have had higher education. They were also less likely to have had a previous transfusion or cardiac surgery.

Table 2 summarises the observed post-transfusion hepatitis events over the total time, and the results of the Mantel-Haenszel analysis comparing the no-withhold with the withhold group. This method of analysis combined the data from the two time periods (table 3) by averaging the two period specific odds ratios (one sided p=0·065 for overall post transfusion hepatitis). There was no evidence for heterogeneity of the surrogate testing effect between the pre-HCV and post-HCV screening periods at 5% significance, but there was a difference in the post-transfusion hepatitis rates between the two time-periods (Mantel-Haenszel test, p=0·11 for overall post-transfusion hepatitis and p=0·06 for HCV hepatitis, stratifying by centre and group).

There were no cases of HAV or HBV hepatitis in any of the study groups (95% CI, 0 to 1·3 per 1000 in either

	Number of post-transfusion hepatitis events by exposure						
	Unexposed	Exposed			Total exposed	Baseline rate per 1000 in exposed	Relative risk for total exposed
		(1)	(2)	(3)			
Pre-HCV screening (n=799)							
Overall post-transfusion hepatitis	6	1	1	2	4	8·2	7·3 (p<0·01)
HCV hepatitis	1	1	1	2	4	1·4	43·4 (p<0·01)
non-ABC hepatitis	5	0	0	0	0	6·9	*
Subjects at risk	729	42	24	4	70		
Post-HCV screening (n=3793)							
Overall post-transfusion hepatitis	25	2	2	0	4	7·1	2·3
HCV hepatitis	7	1	0	0	1	2·0	2·0
non-ABC hepatitis	18	1	2	0	3	5·1	2·4
Subjects at risk	3540	143			91	19	253

Unexposed includes the group that received units with alanine aminotransferase and did not have antibodies ≤40. Exposed (1) includes at least one unit alanine amino transferase above 40 units and all units anti-HBc negative; exposed (2) includes at least one unit anti-HBc positive and all units with alanine aminotransferase less than 40 units; exposed (3) includes at least one unit with alanine aminotransferase >40 IU/L and at least one unit anti-HBc positive.
*Estimates of relative risk fornon-A, non-B, non-C (non-ABC) post-transfusion hepatitis in the pre-HCV screening period are unreliable because of absence of events, but the exact score test was non-significant (p=1·00).

Table 4: **Number of post-transfusion hepatitis events by exposure status**

study group for either outcome). Over the entire period, the combined rate for HCV hepatitis was 4·4 in the no-withhold group and 1·3 in the withhold group (p=0·048), whereas the non-A, non-B, non-C hepatitis rates were 6·1 and 5·2, respectively (p=0·65). Furthermore, this rate was similar to that of the syngeneic blood-product recipients—namely, 6·1 (95% CI, 1·7 to 15·6). No HCV hepatitis was seen in the syngeneic blood recipients (95% CI, 0 to 4·6 per 1000).

Impact of anti-HCV screening on the benefit of NANB surrogate markers

We also analysed our data according to whether HCV screening was in place (table 3). Before HCV screening, the overall post-transfusion hepatitis rate per 1000 subjects was 20·2 in the no-withhold group compared with 5·0 in the withhold group (p=0·05). After mandatory HCV screening, the overall post-transfusion hepatitis rates were 8·6 and 6·8, respectively (p=0·55). The estimated benefit of NANB surrogate testing was 75% before HCV testing (95% CI, −15 to 95%), and 20% after HCV testing (−67 to 62%).

Before HCV screening NANB surrogate testing had an effect on HCV hepatitis (p=0·06) but not on non ABC hepatitis (p=0·98). After screening for HCV because of requirement, the effects on HCV hepatitis and non-A, non-B, non-C hepatitis were smaller and non-significant (p=0·71 and 0·97, respectively). The estimated benefit of NANB surrogate testing for HCV hepatitis was 85% before HCV screening (95% CI, −7% to 100%), and dropped to 41% after HIV screening (−200 to 91%). Homogeneity of effects over time was rejected (two-sided p=0·04, using a pooled withhold group).

Explanatory analysis

Table 4 summarises the number of post-transfusion hepatitis events by the surrogate marker status of the donor units received, and gives estimates of the relative risk of post-transfusion hepatitis before and after HCV screening.

Among the 799 subjects who received blood before the HCV screening period, 70 (8·8%) had received at least one unit positive for either NANB marker. Among the 3793 recipients of blood after HCV screening, 253 (6·7%) had had surrogate-marker-positive units. In recipients of at least one NANB-marker-positive unit, the risk of HCV hepatitis decreased significantly from 57·1 per 1000 recipients before HCV screening to 4·0 per 1000 after HCV screening (p=0·02). The multivariate regression analyses indicated that the alanine aminotransferase and before HBc markers acted independently, and the relative-risk estimates specific to each marker were similar whether the other marker was included in the regression model or not. Tests for homogeneity over time suggested that the relative risk of HCV hepatitis with exposure to NANB surrogate-marker-positive units was lower after HCV screening, especially for the HBc marker, although the significance was marginal.

The per recipient rate of overall post transfusion hepatitis and HCV hepatitis in the no withhold groups increased significantly with the number of positive units transfused, but the numbers of units that were negative for the NANB markers was not associated with the risk of disease.

The per unit rate of HCV hepatitis dropped from 4·2 per 1000 units in the no-withhold group to 0 in the withhold group before HCV screening and from 0·6 to 0·4 per 1000 after HCV screening. However, because there was no dose-response relation in the total number of units transfused, the per unit rates cannot be used to estimate individual risk.

Discussion

During 1984–85, Feinman et al[1] reported an overall incidence of NANB hepatitis of 92 per 1000 allogeneic blood product recipients. Subsequently, about one-third (31 per 1000) of these allogeneic blood recipients developed HCV hepatitis.[2]

During our study, withholding of NANB surrogate marker positive units reduced the overall post-transfusion hepatitis rate by 40%. Most of the benefit of NANB surrogate testing was due to a reduction in HCV hepatitis before blood was screened for HCV. During the period when screening for HCV was a requirement, the statistical power of our sample size was too low to detect moderate effects of NANB surrogate testing because of the extremely low event rate. The introduction of HCV screening thus appears to have modified the effect of NANB surrogate testing by reducing the risk of HCV hepatitis associated with exposure to NANB surrogate-marker-positive units. Nonetheless, our data suggest that NANB surrogate testing in Canada before May, 1990,

would have reduced the frequency of NANB hepatitis, especially that caused by HCV. By contrast, since HCV screening is now mandatory, the benefits of NANB surrogate marker testing are not supported by our data. Furthermore, the introduction of second and third generation HCV screening will probably decrease the HCV hepatitis risk further.[14]

The drop in the HCV hepatitis rate from 31·3 per 1000 to 12·6 per 1000 between 1984–85 and 1988–90 appears to have been associated with improved methods for the screening of blood donors, since the drop occurred without NANB surrogate markers. In the USA a similar reduction in HCV hepatitis was reported over the same period in association with the introduction of NANB surrogate marker testing.[15,16]

Our study provides an interesting perspective on the potential nature of non ABC hepatitis. In at least 2 subjects cytomegalovirus was the causal agent. While it is possible that other non ABC hepatitis cases may have been caused by a hitherto unknown virus, from our study it appears that neither the introduction of anti-HCV screening nor the use of NANB surrogate markers had an impact on the frequency of non ABC hepatitis. Interestingly, 6·1 per 1000 recipients of syngeneic blood products had increases in alanine aminotransferase compatible with a diagnosis of this type of hepatitis. While hepatitis may be due to other viruses or to biologically active products present in stored blood (syngeneic or allogeneic), we believe that most cases with non ABC hepatitis are not transfusion related at all.

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References

1 Feinman SV, Berris B, Bojarski S. Post-transfusion hepatitis in Toronto, Canada. *Gastroenterology* 1988; **95:** 464–69.

2 Feinman SV, Berris B, Herst R. Anti-HCV in post-transfusion hepatitis: deductions from a prospective study. *J Hepatol* 1991; **12:** 377–81.

3 Dodd RY. The risk of transfusion-transmitted infection. *N Engl J Med* 1992; **327:** 419–21.

4 Chiavetta JA, Nusbacher J, Wall A. Donor self-exclusion patterns and human immunodeficiency virus antibody test results over a twelve-month period. *Transfusion* 1989; **29:** 81–83.

5 Aach RD, Szmuness W, Mosley JW, et al. Serum alanine aminotransferase of donors in relation to the risk of non-A non-B hepatitis in recipients: the transfusion-transmitted viruses study. *N Engl J Med* 1981; **304** 989–94.

6 Koziol DE, Holland PV, Alling DW, et al. Antibody to hepatitis B core antigen as a paradoxical marker for non-A non-B hepatitis agents in donated blood. *Ann Intern Med* 1986; **104:** 488–95.

7 Choo QL, Richman KH, Han JH, et al. Genetic organization and diversity of the hepatitis C virus. *Proc Natl Acad Sci USA* 1991; **88:** 2451–55.

8 Wright TL, Mamish D, Combs C, et al. Hepatitis B virus and apparent fulminant non-A non-B hepatitis. *Lancet* 1992; **339:** 952–55.

9 Feinman SV, Berris B, Fay O, et al. Serum HBV-DNA (Hepatitis B virus DNA) in acute and chronic hepatitis B infection. *Clin Invest Med* 1988; **4:** 289–91.

10 Bukh J, Purcell RH, Miller RH. Importance of primer selection for the detection of hepatitis C virus RNA with the polymerase chain reaction assay. *Proc Natl Acad Sci USA* 1992; **89:** 187–91.

11 Gail M. The determination of sample szes for trials invollving several independent 2×2 tables. *J Chron Dis* 1973; **26:** 669–73.

12 Geller NL, Pocock SJ. Interim analysis in randomized clinical trials: ramifications and guidelines for practitioners. *Biometrics* 1987; **43:** 213–23.

13 Begg CB, Gray R. Calculation of polychotomous logistic regression parameters using individualized regressions. *Biometrika* 1984; **71:** 11–18.

14 Kleinman S, Busch M, Holland P. Post-transfusion hepatitis C virus infection. *N Engl J Med* 1992; **327:** 1601.

15 Donahue JG, Munoz A, Ness PM, et al. The declining risk of post-transfusion hepatitis C virus infection. *N Engl J Med* 1992; **327:** 369–73.

16 Nelson KE, Ahmed F, Ness P, Donahue JG. The incidence of post-transfusion hepatitis. *N Engl J Med* 1993; **328:** 1280–81.