A novel study of association between *Neisseria gonorrhoeae* and the human carbohydrate blood groups

H.E. Perry, R.A. Franklin, S.J. Bray, M.K. Lo, L.A.C. Svensson, and S.M. Henry

Previous studies of association of ABO blood groups with gonorrhea have shown contradictory results. Despite the interdependencies of ABO, Lewis, and secretor systems, none of the previous studies examined the combined effect of these systems on their proposed association with gonorrhea. This study attempted to redress that and used genotyping in addition to RBC phenotyping to determine correct tissue phenotypes. Samples from 131 gonorrhea-positive individuals and from 175 gonorrhea-negative individuals were typed for ABO and Lewis using routine antisera. Secretor and Lewis genotyping was performed to ensure accurate determination of ABO and Lewis phenotypes. Chi-square and probability values were used to examine whether there is an association of ABO, Lewis, and secretor systems with gonorrhea infection. Neither single nor combined statistical analysis of data sets yielded a significant association of ABO, Lewis, and secretor phenotypes with *Neisseria gonorrhoeae*. Nevertheless, this study is an example of the approach that should be taken when examining microbial associations with ABO antigens, in turn influenced by coexpression and modification by the interdependent systems of Lewis and secretor, in mucosal tissues. ***Immunohematology*** **2007; 23:100–104.**

This study examines whether there is an association between gonorrhea infection and the carbohydrate blood groups. Some microorganisms are known or believed to use specific carbohydrate receptors to invade the human mucosa.[1-4] Although the precise binding mechanism of microorganisms to mucosa is not well understood, it is probable that carbohydrate ligands such as those determined or modified by the blood group systems of ABO, secretor, and Lewis are involved.[5] Earlier studies have examined possible relationships between blood groups and gonorrhea, but are contradictory. Three separate studies reported gonorrhea to be more common in group B individuals.[6-8] However, three other similar studies found no relationship between ABO antigens and gonorrhea.[9-11]

Expression of ABO antigens in tissues is more complex than the simple expression of ABO antigens on RBCs.[12,13] Both Lewis and secretor genes have a major influence on the expression of blood group structures in mucosal tissues. Furthermore, it is established but poorly appreciated that typing RBCs is an inaccurate way of determining the Lewis phenotype of tissues. The reason for this is both the poor quality of Lewis reagents and the preference for conversion of precursor into ALe^b and BLe^b rather than Le^b in group A and B individuals.[5,12]

Consequently, determining the phenotype of RBCs alone cannot accurately predict the expression of the ABO and Lewis antigens in tissues. Determining the genetic status of the individual, as performed in this study, determines "true" tissue phenotypic expression of ABO, Lewis, and secretory antigens.

Table 1 summarizes the relative expression of antigens expected in mucosal tissues in some genotypes and phenotypes relevant to, and tested in, this study.

Materials and Methods

Attendees at Auckland Sexual Health clinics were invited to participate in the study (ethical approval; Auckland Ethics Committees 2001/113). The cohort consisted of 131 individuals who tested positive for gonorrhea (G+) at the District Health Board (DHB) laboratory, and 175 individuals who tested negative for gonorrhea (G–) at the DHB laboratory. Ethnicity was self-determined by participants into five groups (Maori, Pacific Island, Asian, New Zealand European, and other) and then coded by number so that ethnicity was known only by code (as required by the ethics committee).

Table 1. Constructed using available and unpublished data to compare and contrast relative expression of molecules on the tissues, and in the secretions, of individuals with different ABO, Le, and Se genetic profiles

	Relative antigen expression in secretions and mucosal surfaces*							
Genes present	**Le^c**	**Le^d**	**Le^a**	**Le^b**	**A-1/B-1**	**ALe^b**	**BLe^b**	**RBC phenotype**
A, Le, Se	(+)	(+)	+	++	++	++++		A Le(a–b+)
B, Le, Se	(+)	(+)	+	++	++		++++	B Le(a–b+)
O, Le, Se	(+)	+	+	++++++				O Le(a–b+)
ABO, Le, sese	+		++++++					ABO Le(a+b–)
A or *B, lele, Se*	(+)	++			+++++			A or B Le(a–b–)
O, lele, Se	(+)	++++++						O Le(a–b–)
ABO, lele, sese	++++++++							ABO Le(a–b–)

* Le^c = type 1 precursor; Le^d = H type 1; A-1/B-1 = either A type 1 or B type 1
(+) Very low expression
+, ++ Low expression
++++, +++++, ++++++ Moderate expression
++++++++ High expression

One 10-mL tube of blood, anticoagulated with CPD, was collected from each participant. Blood samples were centrifuged at 1000 × *g* for 20 minutes to separate plasma, WBCs, and RBCs. ABO and Lewis typing and DNA extraction were performed on the centrifuged sample. ABO RBC and plasma blood grouping with commercial antisera (monoclonal anti-A and anti-B, Commonwealth Serum Laboratories, Australia) and reagent RBCs was performed by standard saline tube method. Serologic Lewis typing was performed with commercial antisera (monoclonal anti-Le^a and anti-Le^b, Ortho-Clinical Diagnostics, Raritan, NJ) and unwashed RBCs by a standard saline tube method. Lewis genotyping was performed on all samples with Le(a–b–) phenotypes. Secretor status was determined by genotyping.

Extracted DNA was subjected to PCR amplification for genotyping. Secretor genotyping was performed using an established method capable of detecting a range of mutations including Se^w.[14] Participants were classified as either "secretor," "nonsecretor," or "partial secretor." Lewis genotyping by an established PCR sequence-specific primer method[15] was undertaken on samples from 32 individuals whose RBCs typed as Le(a–b–). The Lewis-negative status was assigned to samples proven to lack *Le* by genotyping.

Phenotypes reported and used for analyses were based on a combination of serologic phenotypes and supportive genotypes. Where there was a discrepancy between the RBC phenotype and the genotype, the genotype took preference.

Chi-square and probability values were used to examine whether there is an association of ABO, Lewis, and secretor blood group–related phenotypes with gonorrhea infection, both in isolation and in a set of five hypotheses based on expression of different carbohydrate molecules, as a result of the different gene combinations. The hypotheses are that Le^a, Le^b, Le^c, Le^d, and ABO antigens are predisposing factors to infection with *Neisseria gonorrhoeae.*

Results

The characteristics of the study cohort are shown in Table 2. As previously reported,[16] there is a significant statistical difference between ethnic distribution in the G+ and G– groups ($p < 0.001$).

ABO, Lewis, and secretor genotypes were determined for all individuals (Table 3). A simple comparison of the association of G+ with the RBC-defined ABO group revealed no statistically significant difference between ABO blood groups and gonorrhea infection alone ($p = 0.95$; Table 3). As individuals that type as group B have been reported to

Table 2. The study cohort

	G+ group	**G– group**
Gonorrhea status	Positive	Negative
Number of individuals	131	175
Age (years)	14-60	15-64
Male	93	125
Female	36	49
Gender not stated	2	1
Ethnic group 1	21	7
Ethnic group 2	59	123
Ethnic group 3	5	19
Ethnic group 4	35	17
Ethnic group 5	6	8
Ethnicity not stated*	5	1

*Participants not used for statistical analysis requiring ethnicity.

Table 3. ABO, Lewis, and secretor distribution in G+ and G– groups (all ethnicities)

	ABO				Secretor			Lewis			
	A	B	AB	O	Se	NS	Se^w	a–b+	a+b+	a+b–	a–b–
G+	52 (40%)	15 (11%)	7 (5%)	57(44%)	75 (57%)	21 (16%)	35 (27%)	74 (56%)	33 (25%)	16 (12%)	8 (6%)
G–	64 (36%)	21 (12%)	10 (6%)	80 (46%)	113 (65%)	35 (20%)	27 (15%)	109 (62%)	26 (15%)	27 (15%)	13 (7%)
χ^2	0.31				6.0			5.31			
p	0.95				0.05			0.15			

Se = secretor (genotypes *SeSe, SeSe^w, Sese*); NS = nonsecretor (genotype *sese*); Se^w = partial secretor (genotypes *Se^{w}Se^w, Se^{w}se*)

be linked with gonorrhea infection in previous studies,[6-8] the significance of an individual typed as group B was reexamined. Group B individuals, alone or when combined with group AB individuals, were tested against individuals of all other blood types, and the results were not significant (p = 0.68 and p = 0.58, respectively).

There was no statistically significant difference between RBC Lewis phenotypes and gonorrhea status (p = 0.15; Table 3).

The relationship between secretor status in the G+ and G– cohorts initially showed statistically significant differences related to the partial secretor phenotype (p = 0.05; Table 3). However, as the *Se^w* gene is not expressed in ethnic group 2, the data were reanalyzed in Se^w-expressing ethnic groups and there was no statistical significance (p = 0.33).

None of the results of hypotheses 1, 2, 3, and 5 showed any association between gonorrhea and combinations of blood group expression on the mucosal tissues (Tables 4–7). There were insufficient data to test hypothesis 4.

Discussion

Although previous reports in the literature have been ambiguous, the possibility that the microorganism *N. gonorrhoeae* could use carbohydrate blood group antigens as receptors, and thus show a blood group association, is reasonable. Blood group antigen associations have been postulated for many microorganisms, and there is some biochemical evidence to support this.[4,5,17] Regrettably, the determination of blood groups in tissues is much more complex than the simple determination of the RBC phenotype, and many blood group associations have been clouded by incorrect phenotyping.[5] The expression of ABO blood group antigens is controlled by inheritance of ABO genes and the polymorphic secretor gene and modified by the expression of the polymorphic Lewis gene.[12,13] These complex interactions are well known today, yet almost all prior surveys have failed to account for these interactions, which significantly determine the type and quantity of molecules expressed in the tissue of interest (Table 1). The issues of studying a disease and blood group associations are further compounded by the inability to obtain accurate Lewis and secretor genotypes from RBC typing, and racial variations.[18] Error rates in typing for these systems are high,[12] and higher in diseased populations or in some ethnic groups; thus genotyping is essential to determine accurate secretor and Lewis types. The association of a disease with the ABO blood group system can only be determined if the study includes measures to accurately determine ABO, Lewis, and secretor tissue phenotypes.

We determined blood groups and analyzed samples from 131 individuals who were identified as being infected with gonorrhea (G+) against 175 gonorrhea-uninfected (G–) individuals as control subjects. The G– group was selected on the basis of exclusion for gonorrhea positivity, and was therefore not a true randomly selected control group. However, this control group was used rather than published data from blood donor studies because it was believed the control group from the clinic would be a better control of socioeconomic and ethnic factors. It was essential to control for ethnicity in the study, as different ethnic groups have different blood group gene frequencies, and different rates of infection with gonorrhea. Although we were blinded with regard to ethnicity, we were able to compare and contrast the test data with ethnic considerations.

There was as expected[12] a high rate of discordance (17%) between Lewis RBC phenotypes (often used to predict secretor status) and Lewis and secretor genotypes. Genotypes were therefore used for data analysis rather than the serologic phenotypes.

Extensive analysis found no association of G+ infection with blood groups ABO, Lewis, or secretor in ethnic populations based in New Zealand. This study was extensive in that it fully considered the factors related to blood group expression and association with

Table 4. Hypothesis 1. Comparison of high expression of Le^a in all ABO groups with no expression of Le^a in all ABO groups

	ABO Le(a+b–)	vs	ABO and Le(a–b–)
ABO	All		All
Lewis	Positive		Negative
Secretor	Negative		All
G+	16		8
G–	26		12
χ^2	0.021		
p	0.886		

Table 5. Hypothesis 2. Comparison of high expression of Le^b in O Le(a–b+) with no expression of Le^b in all ABO groups

	O Le(a–b+)	vs	ABO + Le(a+b–) and Le(a–b–)
ABO	O		All
Lewis	Positive		Combinations producing no Le^b
Secretor	Positive		
G+	34		24
G–	54		42
χ^2	0.083		
P	0.773		

Table 6. Hypothesis 3. Comparison of high expression of Le^c in Le(a–b–) sese (all ABO groups) with low expression of Le^c in Le(a–b+) (all ABO groups)

	ABO Le(a–b–) sese	vs	ABO Le(a–b+)
ABO	All		All
Lewis	Negative		Positive
Secretor	Negative		Positive
G+	5		73
G–	7		109
χ^2	0.011		
p	0.915		

Table 7. Hypothesis 5. Comparison of expression of A, B, and H in secretors

	A Se	B Se	AB Se	O Se
ABO	A	B	AB	O
Lewis	All	All	All	All
Secretor	Positive	Positive	Positive	Positive
G+	29	7	4	34
G–	33	14	9	57
χ^2	2.294			
P	0.514			

infection. Future studies could be further strengthened if consideration was also given to serotyping or genotyping the microorganisms involved and larger numbers of samples were available. Although blood group associations with disease remain tantalizing, it appears that *N. gonorrhoeae* is not an organism that has an association with ABO, Lewis, or secretor antigens.

Acknowledgments

We are grateful to Neil Binnie and Stuart Young of the School of Applied Mathematics, Auckland University of Technology, for statistical advice, and to Mike Brokenshire of LabPlus, Auckland District Health Board, for technical advice and support.

References

1. Ofek I, Beachey EH. Mannose binding and epithelial cell adherence of *Escherichia coli.* Infect Immun 1978;22:247–54.
2. Cohen M, Anderson D. Genitourinary mucosal defenses. In: Holmes K, Sparling P, Mardh P, eds. Sexually Transmitted Diseases. New York: McGraw Hill, 1999:173–90.
3. Henry SM. Molecular diversity in the biosynthesis of GI tract glycoconjugates. A blood group related chart of microorganism receptors. Transfus Clin Biol 2001;8:226–30.
4. Boren T, Falk P, Roth KA, et al. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science 1993; 262:1892–5.
5. Henry SM, Samuelsson B. ABO polymorphisms and their putative biological relationships with disease. In: King, MJ, ed. Human Blood Cells: Consequences of Genetic Polymorphisms and Variations. London: Imperial College Press, 2000: 15–103.
6. Kinane DF, Blackwell CC, Winstanley FP, et al. Blood group, secretor status, and susceptibility to infection by *Neisseria gonorrhoeae.* Br J Vener Dis 1983;59:44–6.
7. Foster MT, Labrum AH. Relation of infection with *Neisseria gonorrhoeae* to ABO blood groups. J Infect Dis 1976;133:329–30.
8. Miler JJ, Novotny P, Walker PD, et al. *Neisseria gonorrhoeae* and ABO isohemagglutinins. Infect Immun 1977;15:713–9.
9. Johnson AP, Osborn, MF, Hanna NF, et al. A study of the relationship between ABO blood groups,

secretor status and infection with *Neisseria gonorrhoeae.* J Infect 1983;6:171–4.
10. Matzkin H. Epidemiology of gonorrhea among men in the Israel Defense Forces. Isr J Med Sci 1987;23:249–51.
11. Schofield CB. ABO and Rhesus blood group distribution among patients attending venereal diseases clinics. J Med Genet 1966;3:101–3.
12. Henry S, Oriol R, Samuelsson B. Lewis histo-blood group system and associated secretory phenotypes. Vox Sang 1995;69:166–82.
13. Oriol R. ABO, Hh, Lewis and secretion. Serology, genetics and tissue distribution. In: Cartron JP, Rouger, P, eds. Blood Cell Biochemistry. New York: Plenum Press, 1995:37–73.
14. Henry SM, Benny AG, Woodfield DG. Investigation of Lewis phenotypes in Polynesians; evidence of a weak secretor phenotype. Vox Sang 1990;58: 61–6.
15. Svensson L, Petersson A, Henry SM. Secretor genotyping for A385T, G428A, C571T, C628T, 685delTGG, G849A and other mutations from a single PCR. Transfusion 2000;40:856–60.
16. Grahn A, Elmgren A, Aberg L, et al. Determination of Lewis FUT3 gene mutations by PCR using sequence-specific primers enables efficient genotyping of clinical samples. Hum Mutat 2001; 18:358–9.
17. Turley M, McNicholas A, Nesdale A, et al. Sexually transmitted infections at New Zealand sexual health clinics, 1999. New Zealand Public Health Report 2000;7:49–52.
18. Mourant AE, Kopec A, Domaniewska-Sobczak K. The Distribution of Human Blood Groups and Other Polymorphisms. Oxford: Oxford University Press, 1976.

Holly E. Perry, MAppSc (Hons), (corresponding author), Faculty of Health and Environmental Sciences, AUT University, Private Bag 92006, Auckland 1142, New Zealand; Rick A. Franklin, FACSHM, Auckland Sexual Health, Auckland District Health Board, Auckland, New Zealand; Susan J. Bray, MBChB, Waikato Sexual Health, Waikato District Health Board, Hamilton New Zealand; and Min K. Lo, MBChB, Auckland Sexual Health, Auckland District Health Board, Auckland, New Zealand; Lola A.C. Svensson, Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden; and Stephen M. Henry, PhD, Faculty of Health and Environmental Sciences, AUT University, Aukland, New Zealand.

Phone, Fax, and Internet Information: If you have any questions concerning *Immunohematology, Journal of Blood Group Serology and Education,* or the *Immunohematology Methods and Procedures* manual, **contact** us by e-mail at immuno@usa.redcross.org. For information concerning the National Reference Laboratory for Blood Group Serology, including the American Rare Donor Program, please contact Sandra Nance, by phone at (215) 451-4362, by fax at (215) 451-2538, or by e-mail at snance@usa.redcross.org