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Immunoglobulins and complement factor C4 in adult rhinosinusitis

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Summary

We assessed whether complement and its factor C4 or abnormal immunoglobulin levels are associated with chronic or recurrent rhinosinusitis. We used multiple patient and control groups to obtain clinically meaningful data. Adult chronic or recurrent rhinosinusitis and acute purulent rhinosinusitis patients were compared with unselected adults and controls without previous rhinosinusitis. Associated clinical factors were reviewed. Levels of immunoglobulins, plasma C3, C4 and classical pathway haemolytic activity were analysed. C4 immunophenotyping was used to detect C4A and C4B deficiencies as null alleles. Complement was up-regulated in rhinosinusitis. C4A nulls and low IgA, IgG, IgG1, IgG2, IgG3 and IgG4 levels were all more common in chronic or recurrent rhinosinusitis patients than in unselected and healthy controls. We searched for relevant differences between the patient groups. According to stepwise logistic regression analysis, nasal polyposis [odds ratio (OR) 10·64, 95% confidence interval (CI) 2·5–45·7, $P = 0{\cdot}001$], bronchial asthma (OR 8.87, 95% CI 2·3–34·9, $P = 0{\cdot}002$), C4A null alleles (OR 5·84, 95% CI 1·4–24·9, $P = 0{\cdot}017$) and low levels of IgG4 together with either IgG1 or IgG2 (OR 15·25, 95% CI 1·4–166·8, $P = 0{\cdot}026$) were more common in chronic or recurrent rhinosinusitis than in acute rhinosinusitis patients. Isolated low IgG subclasses had limited value in patient assessment. C4A null alleles are associated with chronic or recurrent rhinosinusitis, potentially through their effect on immune defence and inflammation control. Multiple clinical and immunological parameters may need to be evaluated when searching for prognostic variables.

Keywords: complement, disease susceptibility, immunoglobulins, MHC/HLA, mucosal immunity, polymorphisms, resistance

Introduction

Chronic rhinosinusitis has a prevalence of 2% and causes a decrease in the quality of life similar to rheumatoid arthritis [1]. The wide range of infectious and inflammatory disorders of sinuses makes them common in general practice [2]. The immunological mechanisms leading to complicated sinus infections remain enigmatic.

Immunoglobulin deficiencies predispose subjects to treatment-refractory chronic or recurrent rhinosinusitis (CRRS). IgA and IgG2 deficiencies in children may remain asymptomatic or progress to severe common variable immunodeficiency (CVID) [3,4]. In adult CRRS, low IgG4 levels are seen only occasionally. The frequency of diminished vaccination responses is not established [5,6]. Patients with one or more subclasses absent are usually asymptomatic. Those with low serum subclass levels often have elevated levels of other subclasses [7]. Decreased levels are caused mainly by regulatory disorders of antibody class-switching [8,9]. In adult CRRS, mainly the levels of complement-fixing IgG1 and IgG3 subclasses are low [5,10–16]. Frequencies of low immunoglobulin levels in the general population and acute rhinosinusitis patients are unknown, making clinical evaluation difficult [4,7].

Complement participates in opsonization, chemotaxis, leucocyte activation and direct killing of microbes [17]. It augments antibody formation and the development of immunological memory [18]. Recurrent rhinosinusitis is

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associated with classical complement pathway component C2 (C2) deficiency [19].

C4 is an early factor of the classical pathway [17]. It is encoded by the C4A and C4B loci in the major histocompatibility complex (MHC) class III area, close to proposed susceptibility genes of IgA deficiency and CVID [20,21]. One to four genes may be present in an MHC haplotype [21]. In Caucasians, over two-thirds of the haplotypes have two genes. The presence of only one C4A or C4B gene in a diploid genome manifests as a low level of the corresponding C4 protein, called a null allele (C4Q0). Null alleles may be caused by gene deletions, point mutations or conversions [21].

The classical pathway is triggered by antigen–antibody complexes. The C4A allotype binds pathogens carrying amino groups [21]. It is an efficient anti-inflammatory scavenger molecule and clears immune complexes and apoptotic cells [17,22]. Accordingly, C4A deficiency may be perceived either as a state of immunodeficiency or hyperinflammation. C4A and C4B propagate the classical and mannose-binding lectin pathways. C4B binds pathogens that express carboxy groups on their surface [21]. C4 nulls are associated with bacterial and viral infections [23–29]. Complete C4A deficiency is present in 3% and C4B deficiency in 8% of the population. Up to 35–40% of individuals have at least one null allele, making C4Q0 probably the most common deficit of immunity [21,30].

C4 nulls have not been studied in rhinosinusitis patients. In a four-arm case–control setting, we studied the frequencies of C4A and C4B nulls and lowered plasma immunoglobulins in severe chronic or recurrent, and in acute rhinosinusitis patients, in an unselected adult population and in subjects without previous rhinosinusitis.

Materials and methods

Study populations

We recruited 55 successive chronic or recurrent rhinosinusitis patients admitted to the Division of Infectious Diseases, Helsinki University Central Hospital between March 1996 and March 2001, typically because of suspected immunodeficiency or planned long-course antibiotic therapy. They had to satisfy the published diagnostic criteria and have no clear response to sinonasal surgery (other than septoplasty), repeated short-course antibiotics and maximal topical medical management [31]. Seven patients could not be included (two refused to participate, two died before inclusion, one had small vessel vasculitis, one was pregnant and one had a known secondary immunodeficiency, HIV). The remaining 38 patients formed the CRRS group. During 9 months in four time-periods between February 2001 and June 2002, we recruited 50 consecutive voluntary patients into the acute rhinosinusitis (ARS) group from the Vihti Municipal Health Centre suffering from acute uncomplicated purulent rhinosinusitis (< 4 yearly episodes, and no previous rhinosinusitis episodes lasting > 3 months). Verified by an otorhinolaryngologist, all had symptoms lasting over 7 days and either fluid level or opacity in a sinus radiograph ($n = 39$) or purulent discharge in sinus puncture with lavage ($n = 12$). The healthy control group comprised 48 subjects age- and sex-matched to CRRS patients from 100 voluntary blood donors with no self-reported history of rhinosinusitis that would fulfil the published criteria [31]. The unselected control group comprised 150 voluntary subjects coming to Vita Laboratory Ltd for a health survey before accepting a new occupational post (Table 1).

Laboratory methods

If not discussed in more detail, all analyses were performed according to the manufacturers' instructions, or as referenced. Plasma IgA, IgM, IgG (Dade Behring BN ProSpec, Marburg, Germany) and IgG1–4 (PeliClass BN, Sanquin Reagents, Amsterdam, the Netherlands) were measured by nephelometry. We used the manufacturer's reference values for levels below two standard deviations from the mean to define low immunoglobulin levels. For seven CRRS patients receiving permanent immunoglobulin substitution, historical values of IgA, IgM, IgG and IgG1–4 were used. Their complement analyses were performed 4 weeks after previous administration of immunoglobulin [32]. CRRS patients generally had multiple measurements available. Mean IgA, IgM, IgG and IgG1–4 values, calculated from the highest and lowest values during clinical follow-up, were used.

Plasma concentrations of C4, C3 were analysed by nephelometry (Behringwerke AG, Marburg/Lahn, Germany), and serum classical pathway haemolytic activity by an enzyme-linked immunosorbent assay (ELISA) technique (CH50; Quidel Corporation, San Diego, CA, USA). CH50 above 200 IU/ml was coded as 200. Allotyping of C4A and C4B proteins was performed electrophoretically from

Table 1. Study populations.

	Chronic or recurrent rhinosinusitis ($n = 48$)	Acute rhinosinusitis ($n = 50$)	Unselected general population ($n = 150$)	Healthy blood donors ($n = 48$)
Age[a]	41·4 (20–68)	42·9 (18–83)	33·7 (18–60)[b]	41·7 (19–65)
Male/female	15/33	11/39	49/101	15/33

[a]Data are expressed as mean (range) in years, [b]in average 8·3 years younger than in other groups, $P \leq 0{\cdot}001$, *t*-test.

carboxypeptidase B (Roche Diagnostics Gmbh, Mannheim Germany) and neuraminidase (Sigma-Aldrich Chemie Gmbh, Type IV, Steinheim, Germany) treated serum samples followed by immunofixation with polyclonal anti-C4 antibody (DiaSorin Inc., Stillwater, MN, USA) with the standard procedure [33]. C4A and C4B allotypes were run to specific positions on the gel in relation to the standards [33]. The presence of ≤1 C4A or C4B variants were defined as nulls. We performed C4A genotyping to all 35 samples from CRRS patients with available DNA. The phenotypic C4A nulls from all 14 obtainable samples were confirmed by isotype-specific genomic real-time-polymerase chain reaction (RT–PCR) amplification. Both the probe and reverse primer (Eurogentec, Liege, Belgium) were based on published primer sequences [34]. We used a 6-carboxy fluorescein (FAM)-labelled Scorpions C4A probe and an unlabelled reverse primer according to the manufacturer's instructions with minor modifications (Lokki, manuscript in preparation). C4A pseudogene caused by a 2-base pairs insertion in exon 29 (codon 1213) was analysed by sequence-specific polymerase chain reaction (PCR) [34].

All samples were kept frozen at −70°C. Missing values were excluded (Table 2). All subjects gave written informed consent. The Ethics Committee, Department of Medicine, Hospital District of Helsinki and Uusimaa approved the study protocol.

Clinical factors and their definitions

Using structured questionnaires, the CRRS and ARS patients were interviewed on associated comorbidities and factors (Table 3). In the CRRS group, microbiological findings were recorded. Operative indications, available preoperative computed tomographies (CTs), functional endoscopic sinus surgery (FESS) operations, biopsy reports and perioperative findings were reviewed and scored [31,35,36]. In cases of multiple FESS operations, we used the highest-scoring. In non-eosinophilic histology, no surplus of eosinophils was reported by the pathologist (Table 4).

Nasal polyposis was based on histology, anterior rhinoscopy or perioperative clinical findings. Septal deviation was diagnosed clinically. Bronchial asthma was diagnosed by a specialist, with the right to reimbursable medication through the Social Insurance Institution of Finland. Hypersensitivity to non-steroidal anti-inflammatory agents (NSAIDs) was based on clinical history of provoked, compatible symptoms. Allergy was diagnosed by a previous positive skin-prick test or allergen-specific IgE in serum. Non-allergic rhinitis was defined as recurring or perennial typical inflammatory symptoms to inciting allergens (e.g. pollens, animals, foods) together with negative allergy test results [37]. In irritant rhinitis, the patient had frequent symptoms to non-specific irritants (e.g. fumes, solvents). Immunodeficiency or rheumatic disease was based on published criteria [38,39]. Acute fungal rhinosinusitis, a rare entity in Finland, was not excluded systematically.

Statistical analysis

Differences in proportions between groups were tested by the χ^2 test or Fisher's exact two-tailed test, as appropriate. In continuous variables, comparisons between all groups were performed by nonparametric analysis of variance (Kruskal–Wallis test, Jonkcheere–Terpstra test for ordinal groups), because most variables had non-normal distribution in at least one group. If the variance analysis showed significant differences between groups, the two-sample *t*-test with Bonferroni correction was used to locate them. In the analysis of C4Q0, odds ratios (OR) were calculated using EpiInfo version 6, and they were tested using logistic regression analysis. Forward stepwise logistic regression was used to identify any potential predictors of CRRS from candidate variables in comparisons of CRRS *versus* other study groups. We used the SPSS package for Windows, version 12.0.1.

Results

Study subjects

Subjects in the unselected group were on average 8·3 years younger than in other groups ($P \leq 0{\cdot}001$, *t*-test, Table 1). A correctable cause of rhinosinusitis (smoking, acute molar infection) was more common in ARS patients, while inflammatory conditions and septal deviation were more common in CRRS patients (Table 3). Of CRRS subjects, 31 fulfilled the recently published criteria for chronic rhinosinusitis and 17 had recurrent disease [40]. All had severe disease and multiple comorbidities (Table 4).

Maxillary sinus cultures taken perioperatively or by sinus lavage were positive in 45 CRRS patients (mean number of positive cultures 8·9, range 1–49). The most common pathogens were *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. *Staphylococcus aureus* was cultured repeatedly from 18 patients, 15 of whom had nasal polyposis ($P = 0{\cdot}016$, Fisher's exact test). No chronic fungal infections were found. Of CRRS patients, 26 had had pneumonia (recurrent in 16 patients) and 10 recurrent or secretory otitis media in adulthood.

Complement analyses

Levels of C3, C4 and CH50 had linear trends with the highest values in the ARS group followed by the CRRS, unselected and healthy subjects (Table 2). Only two CRRS patients with C4AQ0 had low plasma C4. Genotyping was concordant in all 14 CRRS subjects with C4AQ0 and DNA available; one subject had the C4A pseudogene. Using multivariate logistic regression analysis, C4AQ0 was more common in CRRS patients in all comparisons (Table 5); the difference was

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Table 2. Plasma and serum values in patients with chronic or recurrent rhinosinusitis (CRRS), acute rhinosinusitis (ARS), unselected population and healthy subjects with no self-reported history of rhinosinusitis.

Value	CRRS (C) $n = 48$	ARS (A) $n = 50$	Unselected (U) $n = 150$	Healthy (H) $n = 48$	Between groups	Linear trend between groups	Concentrations between groups		Values below reference	Values below reference, between groups	
Unit	Median (range)						*P*-value[c]				
Reference range (M/F)	10–90th percentile Values below reference: *n* (%)				*P*-value, global[a]	*P*-value[b]	C vs. A	C vs. U+H	*P*-value, global[d]	*P*-value[e] C vs. A	C vs. U+H
C3	1·05[f] (0·58–1·82)	1·28 (0·93–1·75)	1·01 (0·49–1·58)	0·92 (0·66–1·30)	**< 0·001**	A > C > U > H, **< 0·001**	**< 0·001**	**0·006**	1·000	1·000	1·000
(g/L)	0·86–1·43	1·03–1·59	0·81–1·32	0·76–1·13							
0·5–1·5	0 (0)	0 (0)	1 (1)	0 (0)							
C4	0·19[f] (0·06–0·47)	0·26 (0·09–0·40)	0·19 (0·04–0·34)	0·19 (0·09–0·37)	**< 0·001**	A > C > U > H, **< 0·001**	**< 0·001**	0·738	**0·022**	0·090	1·000
(g/L)	0·13–0·31	0·19–0·33	0·12–0·28	0·12–0·28							
0·15–0·5	8 (17)	2 (4)	32 (21)	10 (21)							
CH50[g]	130[h] (0–200)	198 (75–200)	110 (30–200)	105 (50–175)	**< 0·001**	A > C > U > H, **< 0·001**	**< 0·001**	0·068	0·117	0·102	0·994
(IU/ml)	57–200	126–200	56–175	70–145							
50–130	4 (9)	0 (0)	10 (7)	1 (2)							
IgA	1·71 (0–4·15)	1·84 (0·52–5·15)	1·89 (0–4·35)	1·80 (0·92–4·11)	0·475	–	0·272	0·154	**0·002**	**0·030**	**0·002**
(g/L)	0–2·67	0·98–2·75	0·95–2·79	1·13–2·77							
0·88–4·84/0·52–4·02	8 (17)	1 (2)	5 (3)	0 (0)							
IgM	1·27 (0–2·65)	1·01 (0·44–3·54)	1·11 (0·25–3·82)	1·04 (0·46–5·23)	0·604	–	0·998	1·000	0·118	0·228	0·180
(g/L)	0·49–2·06	0·59–1·81	0·55–1·98	0·70–1·95							
0·36–2·59/0·47–2·84	3 (6)	0 (0)	3 (2)	0 (0)							
IgG	9·78 (0–22·65)	9·25 (4·81–14·50)	11·20 (7·14–17·60)	10·70 (7·94–19·50)	**< 0·001**	H > U > A > C, **< 0·001**	1·000	0·012	**< 0·001**	0·390	**< 0·001**
(g/L)	0·85–14·56	7·25–12·26	8·63–13·59	8·81–12·74							
6·8–15	7 (15)	3 (6)	0 (0)	0 (0)							
IgG1	5·47 (0–17·00)	6·05 (3·44–11·40)	6·60 (2·88–13·30)	5·85 (3·23–10·50)	**< 0·001**	–	0·710	0·128	**< 0·001**	0·044	**< 0·001**
(g/L)	0·33–10·26	4·38–7·95	5·12–8·58	4·84–7·24							
4·9–11·4	18 (38)	8 (16)	10 (7)	6 (13)							
IgG2	2·41 (0–7·95)	2·96 (0·64–6·33)	3·03 (1·39–7·49)	2·98 (1·34–8·52)	**0·004**	H > U > A > C, **0·029**	**0·034**	**< 0·001**	**< 0·001**	0·564	**< 0·001**
(g/L)	0·65–4·02	1·24–4·78	1·98–4·40	1·66–5·04							
1·5–6·4	10 (21)	6 (12)	3 (2)	3 (6)							
IgG3	0·30 (0–2·05)	0·24 (0·10–0·68)	0·32 (0·11–0·84)	0·28 (0·13–0·52)	**0·010**	–	0·344	1·000	**< 0·001**	1·000	**0·002**
(g/L)	0·09–0·68	0·12–0·48	0·18–0·52	0·16–0·42							
0·2–1·1	18 (38)	17 (34)	20 (13)	9 (19)							
IgG4	0·32 (0–2·44)	0·42 (0–3·13)	0·45 (0–2·72)	0·35 (0–1·09)	0·139	–	0·610	0·464	0·080	0·234	**0·022**
(g/L)	0·05–1·14	0·12–1·18	0·12–1·29	0·10–0·83							
0·08–1·4	8 (17)	3 (6)	8 (5)	2 (4)							

M = male, F = female, P = plasma, S = serum, C = complement factor, Ig = immunoglobulin, [a]Kruskal-Wallis, [b]Jonkcheere-Terpstra, [c]two-sample *t*-test with Bonferroni correction for multiple comparisons, [d]Fisher's exact *t*-test, [e]Fisher's exact test with Bonferroni correction, [f]*n* = 46, [g]CH50 = classical pathway haemolytic activity, values ≥ 200 counted as 200. CH50 was measured in serum, other parameters in plasma, [h]*n* = 47, *P*-values <0·05 are in bold type.

Table 3. Comorbidities and clinical findings in patients with chronic or recurrent rhinosinusitis (CRRS) compared with acute rhinosinusitis patients (ARS).

	CRRS ($n = 48$) no. (%)	ARS ($n = 50$) no. (%)	Fisher's exact test *P*-value
Any hypersensitivity	34 (71)	14 (28)	< 0·001
Allergy	15 (31)	7 (14)	0·053
Non-allergic rhinitis	19 (40)	7 (14)	0·006
Nasal polyposis	29 (60)	3 (6)	< 0·001
Bronchial asthma	27 (56)	4 (8)	< 0·001
Non-steroidal anti-inflammatory drug hypersensitivity	14 (29)	0 (0)	< 0·001
Current or previous smoking	13 (27)	29 (58)	0·002
Septal deviation	10 (21)	2 (4)	0·014
Irritant rhinitis	6 (13)	5 (10)	0·471
Upper molar infection	1 (2)	15 (30)	< 0·001
Nasal fractures	0 (0)	3 (6)	0·243

Table 4. Severity classification of patients with severe chronic or recurrent rhinosinusitis.

Characteristics of patients ($n = 48$)	
Operations per patient, including septoplasty, mean no. (range)	4·2 (1–12)
No. (%) of patients with	
Bilateral operations	47 (98)
Functional endoscopic sinus surgery	44 (92)
Antrostomy	31 (69)
Polypectomy	19 (40)
Caldwell–Luc	18 (38)
Other, frontal operations	3 (6)
Absolute indication for surgery	10 (21)
Functional endoscopic sinonasal surgery, mean score (range)[a]	5·7 (2–20)
Computed tomography, mean Lund score (range)[b]	10·6 (0–24)
Perioperative findings, no. patients with	
Discharge[b]: no/purulent/mucous	22/18/7
Mucosa[b]: non-inflamed/inflamed/polypous	11/ 6/30
Histology[c]: non-inflamed/inflamed, non-eosinophilic/eosinophilic	10/20/12

[a]$n = 44$, [b]$n = 47$, [c]$n = 42$.

Table 5. Multivariate logistic regression analysis of C4 deficiencies according to immunophenotyping. Patients with chronic or recurrent rhinosinusitis (CRRS) were compared to acute rhinosinusitis patients (ARS), unselected population, and healthy subjects with no history of rhinosinusitis.

Type of deficiency	No. of isotype proteins	CRRS (*C*) ($n = 48$) no. (%)	ARS (*A*) ($n = 50$) no. (%)	Unselected (*U*) ($n = 150$) no. (%)	Healthy (*H*) ($n = 48$) no. (%)	*C versus A* OR (95% CI) *P*[a]	*C versus U* OR (95% CI) *P*[a]	*C versus H* OR (95% CI) *P*[a]
Any C4A deficiency	≤ 1 C4A	18 (38)	4 (8)	27 (18)	9 (19)	6·9 (2·0–30·1) 0·001	2·7 (1·3–5·6) 0·006	2·6 (0·9–7·5) 0·044
Total C4A deficiency	No C4A	1 (2)	2 (4)	2 (1)	0	n.s.	n.s.	n.s.
Any C4B deficiency	≤ 1 C4B	22 (46)	24 (48)	59 (39)	18 (38)	n.s.	n.s.	n.s.
Total C4B deficiency	No C4B	3 (6)	2 (4)	15 (10)	6 (13)	n.s.	n.s.	n.s.
Any C4 deficiency	≤ 1 C4A or C4B	40 (83)	28 (56)	86 (57)	27 (56)	3·9 (1·4–11·3) 0·004	3·7 (1·6–8·5) 0·003	3·9 (1·4–11·3) 0·005

C = complement factor, CI = confidence interval, OR = odds ratio, n.s. = not significant, [a]*P* values for differences between groups received from logistic regression analysis with chronic as reference group (odds ratios and their confidence intervals calculated with EpiInfo version 6).

greatest between patients with CRRS or ARS [odds ratio (OR) 6·9, 95% confidence interval (CI) 2·0–30·1, $P = 0·001$]. C4AQ0 was not associated with chronic or recurrent disease subtype or any comorbidity. C4AQ0 was not significantly less frequent in ARS patients than in unselected and healthy controls combined ($P = 0·088$, Fisher's exact test).

After study entry, in three CRRS patients with a C4A null in RT–PCR and recurrent, culture-positive rhinosinusitis, we diagnosed systemic lupus (SLE; two with secondary sicca syndrome). One of them had IgA deficiency, one low IgG1 and one low IgG2. Photosensitivity was reported by 12 patients with C4AQ0 and eight with C4BQ0. Patients had no anti-neutrophil cytoplasmic antibodies or anti-nuclear antibodies above the threshold value of 320.

Immunoglobulin concentrations

Plasma IgM, IgA, IgG and IgG subclass concentrations were measured (Table 2). Of CRRS patients, five had CVID and one permanent hypogammaglobulinaemia after non-Hodgkin's lymphoma. IgG and IgG2 concentrations were significantly lower in the CRRS group than in the unselected and healthy controls combined. Only IgG2 concentrations were lower in CRRS than in ARS patients (Table 2).

Frequencies of subnormal immunoglobulin concentrations

No differences in frequencies of subnormal immunoglobulin levels were found between unselected and healthy subjects. Low IgA, IgG, IgG1, IgG2, IgG3 and IgG4 were all more frequent in CRRS patients compared with unselected and healthy controls combined (Table 2). However, when frequencies of low immunoglobulin levels in the CRRS group were compared one by one with the ARS group, only low IgA ($P = 0·030$) and IgG1 ($P = 0·044$) remained significant (Table 2). Subclass levels best differentiating the CRRS patients from those with ARS were the combination of low IgG4 with low IgG1 or IgG2 (eight patients *versus* one, $P = 0·030$, Fisher's exact *t*-test with Bonferroni correction). Selective IgG4 deficiency alone was seen only in groups without CRRS. C4Q0 was not associated with low immunoglobulin or immunoglobulin subclass levels.

Relative contribution of low immunoglobulin levels and C4A nulls

In multiple logistic regression analysis with complement deficiencies and low immunoglobulin levels as independent variables, comparing CRRS patients with unselected and healthy subjects combined, C4AQ0 (OR 2·89, 95% CI 1·3–6·3, $P = 0·007$), low IgG1 (OR 6·23, 95% CI 2·7–14·5, $P = 0·001$), low IgG2 (OR 6·19, 95% CI 1·8–21·0, $P = 0·003$) and low IgG3 (OR 2·38, 95% CI 1·04–5·4, $P = 0·039$) remained significant. Comparing CRRS with ARS patients, only C04AQ0 (OR 7·65, 95% CI 2·3–25·7, $P = 0·001$) and low IgG1 (OR 3·60, 95% CI 1·3–9·9, $P = 0·013$) remained significant.

Relative contribution of all assessed risk factors

Including comorbidity variables (Table 3) into forward stepwise logistic regression analysis, only four factors distinguished CRRS from ARS patients: nasal polyposis (OR 10·64, 95% CI 2·5–45·7, $P = 0·001$), bronchial asthma (OR 8·87, 95% CI 2·3–34·9, $P = 0·002$), C4AQ0 (OR 5·84, 95% CI 1·4–24·9, $P = 0·017$) and low IgG4 together with low IgG1 or low IgG2 (OR 15·25, 95% CI 1·4–166·8, $P = 0·026$, Table 6).

Comparing the CRRS with the ARS group, the CRRS patients more frequently had nasal polyposis, bronchial asthma, C4A null or low levels of IgG4 with IgG1 or IgG2. Having one of these factors differentiated a CRRS patient from an ARS patient with a sensitivity of 87·5% and specificity of 80% rising to 96% with two factors present.

Discussion

We measured frequencies of low IgG subclass levels and C4A nulls from multiple patient and control groups to attain clinically meaningful comparisons. To achieve this, we also assessed patients with uncomplicated disease forms. Although low IgA, IgG1, IgG2 and IgG3 levels were associated with CRRS, isolated low levels had limited value in rhinosinusitis patient assessment. The most frequent factors in plasma associated with chronic or recurrent rhinosinusitis were C4AQ0 and a combination of low IgG4 with low IgG1 or IgG2.

There are no previous data on complement or immunoglobulin levels in acute rhinosinusitis patients or subjects with no previous sinusitis. Population values of subclass levels have been derived from studies on blood donors, who are healthy but may have had previous rhinosinusitis [41]. Using the reference values reported by the manufacturer, we found that decreased subclass levels were common in all our study groups. High frequencies of low subclass values in the general population may compromise the clinical applicability of subclass measurements [7]. Subclass measurements are further perplexed by the lack of universally accepted reference

Table 6. Clinical and immunological risk factors for chronic or recurrent rhinosinusitis group (CRRS), based on forward logistic regression analysis with acute rhinosinusitis group (ARS) as controls.

Risk factor	OR (95% CI)	*P*
Nasal polyposis	10·64 (2·5–45·7)	0·001
Bronchial asthma	8·87 (2·3–34·9)	0·002
Complement factor C4A null	5·84 (1·4–24·9)	0·017
Low immunoglobulin G4 with low G1 or G2	15·25 (1·4–166·8)	0·026

OR = odds ratio, CI = confidence interval.

values and analysis methods. Low IgG1 and IgG3, frequent in all groups, may also be markers of T helper type 2 dominant response. The high number of severe hypogammaglobulinaemias in our CRRS patients is related most probably to the stringent inclusion criteria.

C4 nulls and low subclass levels were not associated in our patient groups [9,42,43]. Total IgG4 deficiency has been found previously together with total C1, C4, C2 and C3 deficiencies, but we found it exclusively in patients without C4A nulls (data not shown) [42,44]. The production of bispecific IgG4 with anti-inflammatory functions is promoted in prolonged, local, complement- and immunoglobulin-mediated inflammation caused by multiple simultaneously occurring antigens [45]. CRRS may fulfil these requirements. Low IgG4 alone was seen mainly in the controls, corroborating previous data [5,11,12,14,15].

Complement was up-regulated in patients. The novel association between CRRS and C4A nulls may be explained by several factors. Complement regulates antibody formation, and is important in first-line innate defence against encapsulated bacteria causing acute rhinosinusitis [18]. Virulent pneumococci and *M. catarrhalis* strains seem to evade complement [46,47]. C4A is involved in the clearance of immune complexes and late apoptotic cells [17,22]. In CRRS, C4A nulls may cause persistence of proteinaceous complexes and inflammation. Plasma IgA and IgG may also have anti-inflammatory functions [22,48,49]. Besides defence, intact humoral immunity may be important in the regulation of inflammation in CRRS.

The frequency of C4BQ0 observed here is the highest reported in general populations. HLA haplotypes carrying C4BQ0 are common in Finland [30]. The frequency of C4BQ0 was similar in all groups. This contrasts with the assumed role of C4B in defence against commonly found encapsulated pathogens. C4 null alleles and gene dose may be underestimated in immunophenotyping [50]. Poor production of C4 can be caused by factors other than C4 gene numbers, but in our study the RT–PCR C4A genotyping results were concordant with the immunophenotyping results [51]. Total C4A deficiency was not more frequent in CRRS. This lack of dose effect may be caused by the small sample size or imply that a nearby gene in strong linkage disequilibrium with C4AQ0 in the MHC is involved [9]. Other MHC genes should be evaluated in the future, e.g. the conserved haplotype B8,DR3 is associated with SLE, C4AQ0 and hypogammaglobulinaemias [9,20]. Chronic hypertrophic rhinosinusitis has been associated with HLA-DQ1*03 [52]. This allele is not carried in haplotypes with C4AQ0. Asthma, allergy and nasal polyposis have associations with MHC, but they did not correlate with C4AQ0. Homozygous C4A4,C4B2 haplotypes found in over 90% of total C2 deficiencies were not observed [19]. The immunologically plausible functions of C4A and degree of statistical significance observed suggest that C4A nulls may predispose to CRRS. Other genes in MHC may have an effect.

Not all our patients fulfil the newly published criteria for chronic disease [40], yet no differences in the measured immunological risk factors between patients with recurrent purulent or chronic rhinosinusitis were found. In treatment-refractory rhinosinusitis, gradual impairment of ciliary function leads to mucus and biofilm accumulation [53]. This impairs local defences and further worsens the clinical status. Hypothetically, immune defences (e.g. C4, IgG1–3, secretory IgA) and anti-inflammatory functions (e.g. C4, IgG4, plasma IgA) may both need to be compromised in CRRS. The relative lack of C4A nulls in the acute rhinosinusitis group supports this. Comorbidities were common in CRRS patients. If better prognostic variables were found, swift immunological evaluation of patients refractory to operative therapy could potentially prevent irreversible damage to mucosa and ciliary function. In our retrospective study, the presence of any one of four factors: nasal polyposis, bronchial asthma, C4A null and low levels of IgG4 together with IgG1 or IgG2, predicted recalcitrance with a sensitivity of 87·5% and specificity of 80%. Our approach will probably be insensitive in selecting patients who will benefit from operative treatment alone.

Plasma IgG subclass measurements had limited value in differentiating patients with recalcitrant rhinosinusitis. C4 typing, either as a marker or as a functional genetic risk factor, performed at least as well. Because the relative contributions of different MHC genes are hard to establish, multiple alleles could be assessed in the future [9]. If further studies corroborate our results, C4 pheno- and genotyping could be used easily as one of several factors in guiding interventions such as vaccines, anti-inflammatory or anti-microbial therapy [54].

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References

1 Shashy RG, Moore EJ, Weaver A. Prevalence of the chronic sinusitis diagnosis in Olmsted County. Minnesota. Arch Otolaryngol Head Neck Surg 2004; **130**:320–3.

2 Cherry DK, Woodwell DA. National ambulatory medical care survey: 2000 summary. Adv Data 2002; **328**:1–32.

3 Lawton AR. IgG subclass deficiency and the day-care generation. Pediatr Infect Dis J 1999; **18**:462–6.

4 Buckley RH. Immunoglobulin G subclass deficiency: fact or fancy? Curr Allergy Asthma Rep 2002; **2**:356–60.

5 May A, Zielen S, von Ilberg C, Weber A. Immunoglobulin deficiency and determination of pneumococcal antibody titers in patients with therapy-refractory recurrent rhinosinusitis. Eur Arch Otorhinolaryngol 1999; **256**:445–9.

6 Edwards E, Razvi S, Cunningham-Rundles C. IgA deficiency: clinical correlates and responses to pneumococcal vaccine. Clin Immunol 2004; **111**:93–7.

7 Lacombe C, Aucouturier P, Preud'homme JL. Selective IgG1 deficiency. Clin Immunol Immunopathol 1997; **84**:194–201.

8 Pan Q, Hammarstrom L. Molecular basis of IgG subclass deficiency. Immunol Rev 2000; **178**:99–110.

9 Larsen CE, Alper CA. The genetics of HLA-associated disease. Curr Opin Immunol 2004; **16**:660–7.

10 Lund VJ, Scadding GK. Immunologic aspects of chronic sinusitis. J Otolaryngol 1991; **20**:379–81.

11 Scadding GK, Lund VJ, Darby YC, Navas-Romero J, Seymour N, Turner MW. IgG subclass levels in chronic rhinosinusitis. Rhinology 1994; **32**:15–9.

12 Armenaka M, Grizzanti J, Rosenstreich DL. Serum immunoglobulins and IgG subclass levels in adults with chronic sinusitis: evidence for decreased IgG3 levels. Ann Allergy 1994; **72**:507–14.

13 Sethi DS, Winkelstein JA, Lederman H, Loury MC. Immunologic defects in patients with chronic recurrent sinusitis: diagnosis and management. Otolaryngol Head Neck Surg 1995; **112**:242–7.

14 Hoover GE, Newman LJ, Platts-Mills TA, Phillips CD, Gross CW, Wheatley LM. Chronic sinusitis: risk factors for extensive disease. J Allergy Clin Immunol 1997; **100**:185–91.

15 Tahkokallio O, Seppala IJ, Sarvas H, Kayhty H, Mattila PS. Concentrations of serum immunoglobulins and antibodies to pneumococcal capsular polysaccharides in patients with recurrent or chronic sinusitis. Ann Otol Rhinol Laryngol 2001; **110**:675–81.

16 Chee L, Graham SM, Carothers DG, Ballas ZK. Immune dysfunction in refractory sinusitis in a tertiary care setting. Laryngoscope 2001; **111**:233–5.

17 Walport MJ. Complement. First of two parts. N Engl J Med 2001; **344**:1058–66.

18 Carroll MC. The complement system in regulation of adaptive immunity. Nat Immunol 2004; **5**:981–6.

19 Jonsson G, Truedsson L, Sturfelt G, Oxelius VA, Braconier JH, Sjoholm AG. Hereditary C2 deficiency in Sweden: frequent occurrence of invasive infection, atherosclerosis, and rheumatic disease. Medicine (Balt) 2005; **84**:23–34.

20 Alper CA, Marcus-Bagley D, Awdeh Z *et al.* Prospective analysis suggests susceptibility genes for deficiencies of IgA and several other immunoglobulins on the [HLA-B8, SC01, DR3] conserved extended haplotype. Tissue Antigens 2000; **56**:207–16.

21 Blanchong CA, Chung EK, Rupert KL *et al.* Genetic, structural and functional diversities of human complement components C4A and C4B and their mouse homologues, slp and C4. Int Immunopharmacol 2001; **1**:365–92.

22 Zwart B, Ciurana C, Rensink I, Manoe R, Hack CE, Aarden LA. Complement activation by apoptotic cells occurs predominantly via IgM and is limited to late apoptotic (secondary necrotic) cells. Autoimmunity 2004; **37**:95–102.

23 Rittner C, Meier EM, Stradmann B *et al.* Partial C4 deficiency in subacute sclerosing panencephalitis. Immunogenetics 1984; **20**:407–15.

24 Rowe PC, McLean RH, Wood RA, Leggiadro RJ, Winkelstein JA. Association of homozygous C4B deficiency with bacterial meningitis. J Infect Dis 1989; **160**:448–51.

25 Bishof NA, Welch TR, Beischel LS. C4B deficiency: a risk factor for bacteremia with encapsulated organisms. J Infect Dis 1990; **162**:248–50.

26 Fasano MB, Densen P, McLean RH, Winkelstein JA. Prevalence of homozygous C4B deficiency in patients with deficiencies of terminal complement components and meningococcemia. J Infect Dis 1990; **162**:1220–1.

27 Cates KL, Densen P, Lockman JC, Levine RP. C4B deficiency is not associated with meningitis or bacteremia with encapsulated bacteria. J Infect Dis 1992; **165**:942–4.

28 Hentges F, Hoffmann A, Oliveira de Araujo F, Hemmer R. Prolonged clinically asymptomatic evolution after HIV-1 infection is marked by the absence of complement C4 null alleles at the MHC. Clin Exp Immunol 1992; **88**:237–42.

29 Pasta L, Pietrosi G, Marrone C *et al.* C4BQ0: a genetic marker of familial HCV-related liver cirrhosis. Dig Liver Dis 2004; **36**:471–7.

30 Laitinen T, Lokki ML, Tulppala M, Ylikorkala O, Koskimies S. Tumour necrosis factor B gene polymorphism in relation to complotype in couples with spontaneous abortions and in control families. Scand J Immunol 1992; **35**:131–5.

31 Lanza DC, Kennedy DW. Adult rhinosinusitis defined. Otolaryngol Head Neck Surg 1997; **117**:S1–7.

32 Lutz HU, Stammler P, Bianchi V *et al.* Intravenously applied IgG stimulates complement attenuation in a complement-dependent autoimmune disease at the amplifying C3 convertase level. Blood 2004; **103**:465–72.

33 Marcus D, Alper CA. Methods for allotyping complement proteins. In: Rose NR, Friedman H, Fahey JL, eds. Manual of clinical laboratory immunology, 3rd edn. Washington, DC: American Society for Microbiology, 1996:185–96.

34 Barba GM, Braun-Heimer L, Rittner C, Schneider PM. A new PCR-based typing of the rodgers and chido antigenic determinants of the fourth component of human complement. Eur J Immunogenet 1994; **21**:325–39.

35 Lund VJ, Mackay IS. Staging in rhinosinusitus. Rhinology 1993; **31**:183–4.

36 Anand VK, Osguthorpe JD, Rice D. Surgical management of adult rhinosinusitis. Otolaryngol Head Neck Surg 1997; **117**:S50–2.

37 Settipane RA, Lieberman P. Update on nonallergic rhinitis. Ann Allergy Asthma Immunol 2001; **86**:494–507.

38 Notarangelo L, Casanova JL, Fischer A *et al.* Primary immunodeficiency diseases: an update. J Allergy Clin Immunol 2004; **114**:677–87.

39 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; **40**:1725.

40 Meltzer EO, Hamilos DL, Hadley JA *et al.* Rhinosinusitis: establishing definitions for clinical research and patient care. J Allergy Clin Immunol 2004; **114**:S155–212.

41 Schauer U, Stemberg F, Rieger CH *et al.* IgG subclass concentrations in certified reference material 470 and reference values for children and adults determined with the binding site reagents. Clin Chem 2003; **49**:1924–9.

42 Bird P, Lachmann PJ. The regulation of IgG subclass production in man: low serum IgG4 in inherited deficiencies of the classical pathway of C3 activation. Eur J Immunol 1988; **18**:1217–22.

43 Gual L, Martinez A, Fernandez-Arquero M *et al.* Major histocompatibility complex haplotypes in Spanish immunoglobulin A deficiency patients: a comparative fine mapping microsatellite study. Tissue Antigens 2004; **64**:671–7.

44 Alper CA, Xu J, Cosmopoulos K *et al.* Immunoglobulin deficiencies and susceptibility to infection among homozygotes and heterozygotes for C2 deficiency. J Clin Immunol 2003; **23**:297–305.

45 Aalberse RC, Schuurman J. IgG4 breaking the rules. Immunology 2002; **105**:9–19.

46 Jarva H, Jokiranta TS, Wurzner R, Meri S. Complement resistance mechanisms of streptococci. Mol Immunol 2003; **40**:95–107.

47 Nordstrom T, Blom AM, Forsgren A, Riesbeck K. The emerging

pathogen *Moraxella catarrhalis* interacts with complement inhibitor C4b binding protein through ubiquitous surface proteins A1 and A2. J Immunol 2004; **173**:4598–606.

48 Cunningham-Rundles C. Physiology of IgA and IgA deficiency. J Clin Immunol 2001; **21**:303–9.

49 Basta M, Van Goor F, Luccioli S *et al.* F(ab)′2-mediated neutralization of C3a and C5a anaphylatoxins: a novel effector function of immunoglobulins. Nat Med 2003; **9**:431–8.

50 Chung EK, Yang Y, Rennebohm RM *et al.* Genetic sophistication of human complement components C4A and C4B and RP-C4-CYP21-TNX (RCCX) modules in the major histocompatibility complex. Am J Hum Genet 2002; **71**:823–37.

51 Yang Y, Chung EK, Zhou B *et al.* Diversity in intrinsic strengths of the human complement system: serum C4 protein concentrations correlate with C4 gene size and polygenic variations, hemolytic activities, and body mass index. J Immunol 2003; **171**:2734–45.

52 Schubert MS, Hutcheson PS, Graff RJ, Santiago L, Slavin RG. HLA-DQB1 *03 in allergic fungal sinusitis and other chronic hypertrophic rhinosinusitis disorders. J Allergy Clin Immunol 2004; **114**:1376–83.

53 Sanclement JA, Webster P, Thomas J, Ramadan HH. Bacterial biofilms in surgical specimens of patients with chronic rhinosinusitis. Laryngoscope 2005; **115**:578–82.

54 Test ST, Mitsuyoshi J, Connolly CC, Lucas AH. Increased immunogenicity and induction of class switching by conjugation of complement C3d to pneumococcal serotype 14 capsular polysaccharide. Infect Immun 2001; **69**:3031–40.

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