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Construction and Clinical Validation of a Questionnaire-based Risk Score to Identify Patients Suffering from Immunodeficiency or Systemic Autoimmunity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HHP and SG designed the questionnaire. Authors SG, JH, HHP, KG, KW, BG and WG collected clinical data. The ISAQ database was established by author SR. Statistical data analysis was performed by authors WV and AN. Authors HHP and WV wrote the manuscript and author BG gave critical input to the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: A self-reporting tool for identifying adults at risk for immune-based diseases was designed and termed immune system assessment questionnaire (ISAQ). It was the aim of this study to validate this novel questionnaire in groups of patients with defined immunodeficiency or autoimmune diseases in comparison to normal adults.
Study Design: Non-randomised, cross-sectional observational study in groups of

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patients and normal adults.
Place and Duration of Study: Department of Rheumatology and Clinical Immunology and Clinical Epidemiology Group, Institute of Medical Biometry and Medical Informatics University Medical Center Freiburg, Germany. Questionnaires from patients and controls were collected between March 2005 and November 2010.
Methodology: Two experienced clinical immunologists selected 17 informative topics including frequency and duration of infections, previous surgery on immunological organs, recent polytrauma, vaccination history, allergies, co-morbidities, use of antibiotics and immunomodulating agents. The ensuing questions were differently weighted based on published evidence and assumed relevance for immunological dysfunction. The questionnaire was distributed to 539 cases suffering from chronic immunodeficiency (n=322) or autoimmunity (n=217) and to 1020 healthy controls. An ISAQ score was calculated for each participant based on the sum of the weighted items.
Results: The median ISAQ-score was 39.5 for cases and 30.4 for controls. 93.7% of all controls scored lower than the median of the cases, which resulted in an AUC of the ROC curve of 0.838 (95%CI: 0.817-0.859). Items with highest predictive value were frequency and duration of infections, use of antibiotics, corticosteroids, immunosuppressants, sinus-mucosa resection, chronic disease of kidney, lungs, gut and hematopoietic system. Although the results indicate that neither the item selection nor the weighting of single items were optimal, several sub-scores with reduced item number could not substitute for the whole ISAQ.
Conclusion: The ISAQ can discriminate between healthy individuals and persons suffering from immune-based disease. The ISAQ performs better for chronic immunodeficiency than for chronic rheumatic diseases; the highest values were obtained for common variable immunodeficiency (CVID) and systemic vasculitides. Future studies are needed to refine item selection and validate the score for predicting immune dysfunction.

Keywords: Questionnaire for immune-based diseases; immunodeficiency; autoimmunity; epidemiology.

ABBREVIATIONS

AUC, area under the curve; ANCA, antineutrophil cytoplasmic antibodies; BMI, body mass index; CVID, common variable immunodeficiency; C/ScD, immunoglobulin class and subclass deficiency; DuID, diverse, undefined immunodeficiency; DMARD, disease-modifying anti-rheumatic drugs; ISAQ, immune system assessment questionnaire; IVIg, intravenous immunoglobulin substitution; RA, rheumatoid arthritis; ROC, receiver operating characteristic. SLE, systemic lupus erythematosous.

1. INTRODUCTION

There exists a large body of knowledge about development, structure, organization and function of the human immune system. The interplay of three main components is essential for its proper function: i. the protecting body surfaces, ii. the innate immune system, which is composed of phagocytes, dendritic cells and NK cells; the innate system senses biological danger by recognizing pathogen associated molecular structures (PAMS) via pattern recognition receptors (PRR, e.g. toll-like receptors), and iii. the adaptive immune system represented by T and B cells, exerting the role of a back-up defence system against viral

and bacterial infections in case the innate system has been overwhelmed by pathogens [1]. Inherited or acquired disturbances of the immune system translate into clinical symptoms whereas as a normally functioning immune system goes asymptomatic [2]. With the exception of a few disease-related immune function scores [3-5], there has been to our knowledge, little attempt to translate symptoms of immune dysfunction into comprehensive questions suitable for a self-assessment questionnaire. Here we present a novel tool for self-assessing the functionality of the immune system. The selected questions address topics based on a long-standing clinical experience of two co-authors (HHP, SG) working for up to 30 years in an immunodeficiency and rheumatology outpatient clinic. The questionnaire was designed to test for the integrity of body surfaces, surgically removed immune organs, lifestyle impacts and chronic use of antibiotics or immuno-suppressive drugs. In addition, the individuals were asked for frequency and duration of infectious episodes, indicator infections, vaccination history and co-morbidities. To test this novel immune system assessment questionnaire (ISAQ) for its ability to identify subjects with increased risk of immune-based diseases, we applied the ISAQ to several cohorts of patients with an established diagnosis of chronic immunodeficiency or autoimmunity (n=539) as well as to different cohorts of healthy controls (n=1020). We observed a distinct difference of the ISAQ scores between cases and controls, indicating that the new tool may be suited to identify individuals at risk for immune-based diseases, particularly immunodeficiency syndromes.

2. MATERIALS AND METHODS

2.1 Recruitment of Patients and Controls

All cases and controls were adults registered in 4 age groups: ≤30, 31 to 45, 46 to 60 and >60 years. They gave informed oral consent to respond in pseudonymized or anonymized form to the ISAQ questionnaire, which was approved by the Ethics Committee of Freiburg University Medical Centre (UMC) (Project Nr. 174/08). With the exception of 79 Sjögren's, 16 SLE and 33 ANCA-positive vasculitis patients who were enrolled during patients' organization reunions, all cases were recruited from the outpatient clinic of the Dept. of Rheumatology and Clinical Immunology of the Freiburg UMC. All patients had an established diagnosis; they were not newly diagnosed but rather under long-term treatment. For patients recruited at the three patients' organization reunions, the diagnoses were self-reported and not verified at our outpatient clinic.

2.1.1 Healthy controls

1020 apparently healthy individuals were recruited at different sites and occasions throughout Germany. The first recruitment was organized in May 2005 during the 11th Health Days of the Dr.ing.h.c.F.Porsche AG, Stuttgart for the employees of the car manufacturer company (P1-workers, n=363). Subsequently, the occupational physicians of the Porsche company (J.H. and K.G.) recruited among employees another 94 controls (P2-workers) who were seen for minor injuries and a third group (P3-workers n=31) who consulted them for common cold infections [6]. Two sampling events were organized in Freiburg at a "Fit-for-life" Fare in 2011 (n=145) and at a University Science Day in 2006 (n=94). Other recruitment sessions were organized during the Annual Meeting of the German Society for Immunology 2007 in Kiel (Kiel DGfI n=33) and a junior immunologist workshop 2009 in Jena (Jena DGfI n=54) as well as at Freiburg UMC (UMC students n=182; UMC employees n=25). Data collection was performed on paper or via an online questionnaire and entered into the ISAQ database hosted at the UMC Freiburg.

2.1.2 Immunodeficiency disorders

2.1.2.1 Common variable immunodeficiency (CVID)

Common variable immunodeficiency (CVID) was the largest and best-characterized cohort of patients enrolled in the study (n= 145). They have been followed between 2005 and 2010 in our CVID outpatient clinic and were classified according to circulating B cell phenotypes [7], distinguishing four subsets of patients (Freiburg classification): All patients suffered from recurrent respiratory or gastrointestinal infections and had low serum immunoglobulin levels of at least two isotypes. In addition, some patients presented with autoimmune phenomena, lymphoproliferation, granulomas and secondary malignancies. CVID Type I exhibits reduced numbers of circulating switched memory B (smB) cells and can be subdivided into a more severe form *Ia* characterized by an increased proportion of $CD21^{low}$ B cells, frequent splenomegaly and autoimmune phenomena and form *Ib* with normal $CD21^{low}$ B cells numbers. CVID type II patients have normal numbers of smB cells and usually exhibit a less severe clinical phenotype. The 4^{th} group of patients is unclassifiable by the Freiburg classification protocol due to the lack of circulating B cells (<1%). Among the 145 CVID patients were also included two patients with defined monogenic defects described for CVID (Baff-R, and TACI deficiency), four with hyper-IgM syndrome (type 1), two Good syndrome patients and one X-linked agammaglobulinemia patient (M.Bruton). All patients were under regular intravenous (IVIg) or subcutaneous (SCIg) immunoglobulin replacement therapy. In a pseudonymized subset of 122 CVID patients composed of 46 type Ia, 46 type Ib, 13 type II and 17 unclassifiable patients the ISAQ was separately analysed and compared among CVID subtypes.

2.1.2.2 Selective IgA, IgM or IgG subclass deficiencies (C/ScD) (n=33)

These patients presented with increased respiratory tract infections and reduced serum level of one or two IgG subclasses and were grouped together as antibody deficiencies without need of IVIg substitution. The group comprised also a patients with selective IgA deficiency (n=4) or low IgM serum concentrations (n=3).

2.1.2.3 Diverse, so far unclassified immunodeficiencies (DuID)

Diverse, so far unclassified immunodeficiencies (DuID) (n=80) which did not fit into the CVID and C/ScD groups but consulted our outpatient clinic for frequent infections of diverse nature, mostly respiratory tract infections. None of them received IVIg substitution therapy.

2.1.2.4 HIV patients

HIV patients (n=64) were recruited from our HIV outpatient clinic; all received highly active anti-retroviral therapy but no IVIg replacement therapy.

2.1.3 Autoimmune disorders

2.1.3.1 Rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) patients (n=43) were recruited from our rheumatology outpatient clinic. They were treated according to current protocols including low-dose corticosteroids, immunosuppressants, and in a minority of the cases also biologicals (Anti-TNFalpha).

2.1.3.2 Systemic lupus erythematosus (SLE) patients

Systemic lupus erythematosus (SLE) patients (n=45) were either seen in our SLE outpatients clinic (n=28) or recruited during a patient organization reunion (n=17). Most of them (>90%) had stable disease under low dose steroids and varying degrees of immunosuppressants (hydroxychloroquine, azathiporpine or mycophenolate mofetil).

2.1.3.3 Sjögren's syndrome patients

Sjögren's syndrome patients (n=87) were mainly (n=79) enrolled during a National Sjögren's Day organized by the German Sjögrens' patient organization in Freiburg 2008. The remaining 8 patients were recruited from our Rheumatology outpatient clinic. Treatment modalities were mainly symptomatic (artificial tears, saliva spray and Salagen®); a minority (<10%) received low-dose steroids and immunosuppressants.

2.1.3.4 Vasculitis patients

Vasculitis patients (n=42) were mainly (n=33) recruited during an Annual Vasculitis Day 2011 organized by a vasculitis patients' organization and the Vasculitis Center of Lübeck/Bramstedt (Head: Prof. W. Gross). The remaining 9 cases were seen at the Vasculitis outpatient clinic of the UMC Freiburg. The majority (>90%) of the patients suffered from ANCA positive vasculitides and were on regular low-dose corticosteroids plus immunosuppressant.

2.2 Item Selection and Composition of the Immune System Assessment Questionnaire (ISAQ)

2.2.1 Process of item selection

The questionnaire for self-assessing the immune system asks for items thought to depend on or influence the function and integrity of the immune system such as frequency and duration of infections, indicator infections (e.g. septicemia, bronchiectasis, recurrent bronchopneumonia, tuberculosis, salmonellosis, HIV, chronic viral hepatitis, meningitis a.o.), vaccination history and co-morbidities. In addition we designed questions to address the integrity of the four body surfaces (skin, respiratory tract, gastrointestinal tract, urogenital tract), recorded surgically removed immune organs, lifestyle impacts, chronic or recurrent use of antibiotics, immunosuppressants, radiation exposure and use of immunostimulants. Table 1 summarizes all items together with the possible response categories. The original German version of the questionnaire is shown in Supplementary Table 1.

Table 1. Immune System Assessment Questionnaire (ISAQ)

Personal ID Number: xxxxxx (6 digits, randomly generated)								
1	Demographic items	Gender	female	male				
		Age (years)	15-30	31-45	46-60	>60		
		Weight (kg)			Height (cm)			
2	Life style items	Smoking: >5 cigarettes/day			n/y	Coffee		n/y
		Alcohol: >0.25l wine, >05l beer			n/y	Drug abuse		n/y
3	Sports activities	n/y	If yes: hrs/week	<1	1-2	3-5	6-10	>10
4	Long distance flights (>4hrs each) per year					<2	2-6	>6

Table 1 Continued……

5	Previous surgery on lymphoid organs			Tonsils	n/y	Sinus mucosa		n/y
				Spleen	n/y	Appendix		n/y
6	Visceral surgery (abdomen, lungs, heart) during the last two years?							n/y
7	Did you ever have a severe accident (polytrauma)?							n/y
8	Vaccinations during childhood?			None	The usual ones			Don't know
9	Which last vaccinations did you receive ? (approximate year)							Unknown
	Diphtheria	year	Hepatitis A/B	year	Tetanus	year	Pneumococci	year
	Influenza	year	Others:					
10	Vaccination-related complications?			none		yes		unknown
11	Infections:	Episodes per year		<1	1-3	4-6		>6
		Duration in weeks		<1	1-2	3		>3
12	Which infections did you suffer from?				a. Upper respiratory tract (otitis,sinusitis)			
	b. Lower respirat.tract (bronchitis, pneumonia)				c. Skin (abscesses, furuncolosis,wound healing)			
	d. GI-Tract (gastritis, diarrhea, IBD)				e. Urogenital (cystitis, pyelitis, prostatitis)			
	f. tooth root infections							
13	Are you HIV positive?		No		Yes	Not tested		No answer
14	Did you suffer in the past from serious infections?					a. Tuberculosis		
	b. sexually transmitted diseases			c.septicemia		d. Salmonellosis		
	e. meningitis			f. tropical diseases		g. Chronic viral hepatitis		
	h. Septic arthritis			i.Others:				
15	Have there been comorbidities diagnosed?				a. Inflammatory rheumatic diseases			
	b. Chronic skin diseases (psoriasis, atopia)				c. Diarrhea, ulcerative colitis, Crohn's disease			
	d. Allergy (Asthma, hay fever, allergic skin disease)				e. Chronic bllod diseases (anemia, thrombocytopenia, leucopenia, lymphopenia)			
	f. Chronic neurological disease (MS, epilepsia)				g. Psychiatric disease (depression, angst a.o.)			
	h. Chronic kidney disease				i. Chronic liver disease			
	j. Chronic lung disease				k. Endocarditis			
	l. Chronic vasculitis (Wegener's, a.o.)				m. Diabetes			
	n. Hypertension, coronary heart disease				o. Malignant tumors			
16	Which drugs do you take regularly?				a. Antibiotics		b. Cortison	
	c. disease modifying anti-rheumatic drugs				d. Immunosuppressants (e.g. azathioprin, MTX)			
	e. cytostatic drugs				f. Immunostimulants			
	g. Intravenous or subcutaneous immunoglobulin							
17	Have you been irradiated in the last 2 years?				No	Yes		Unknown

2.2.2 Construction of the ISAQ score

For the binary items, a negative response got always the weight 0.5, whereas a positive response received the weight 4 or 2, respectively, reflecting whether we regarded the items as more or less important. Items on ordinal or categorical scales were always reduced to a binary item or a trichotomous item. In the latter case the weights 0.5, 2 and 4 were used. All weights are shown in Table 2. The ISAQ score of an individual represents the sum of the weighted items.

2.3 Statistics

As a first test for the potential value of the new tool, we compared the distribution of the single items as well as of the overall score between the patients (termed “cases”) and the controls. To evaluate the value of the single items we considered the prevalence, positive and negative predictive values, sensitivity, specificity, and the odds ratio. The odds ratio is supplemented with a p-value of testing the null hypothesis of no association. For non-dichotomous items we considered the categorization used in the weighting process. If the latter resulted in a trichotomous item, we considered both possible dichotomizations. For some of the categorizations we considered additional dichotomizations. To compare the value of the different items the positive predictive value is plotted against the prevalence. For items with a similar prevalence those with the higher positive predictive value are more useful for inclusion into the score. On the other side, for items with similar positive predictive value, those with the higher prevalence are more useful, as they allow identifying a higher number of patients at risk.

Table 2. Weighting factors

Weighting factors	0	0.5	2.0	4.0
Demographic topics Sex, Age (<30, 31-45, 46-60, >60), Weight, Height	x			
Lifestyle, general risks Smoking (>5 cig/d), Alcohol (>0.25cl wine, >0.5l beer); Coffee, Drug abuse, Sports: (no, <1 h, 1-2h, 3-5h, 6-10h, >10h per week); Frequent Long distance flights (<2, 2-6, >6 per year).	x			
Injuries to the immune system: Surgery of lymphoid organs: tonsillectomy, appendectomy, sinusectomy,			x	
splenectomy				x
General risks				
Major visceral surgery or polytrauma during the last two years (no, yes).		n	y	
Type of vaccinations in childhood : none,			x	
all mandatory vaccinations, don’t know		x		
Vaccinations in the last 5-10 years (diphtheria, tetanus, hepatitis A/B, pneumococcal polysaccarides, influenza)	x			
Symptoms suggestive of ID				
Complications following vaccination (no, yes, unknown).		n, uk	y	
Frequency of infectious episodes/year (<1, 1-3, 4-6, >6).		<1	1-3	4-6, >6
Duration of infectious episodes in weeks (<1, 1-2, 3, >3).		<1, 1-2	3	>3

Abbreviations: n, no; y, yes; uk, unknown

For some items (antirheumatic drugs, immunosuppressants, cytostatic drugs, immunoglobulin) and some patient groups (RA, CVID) a positive response to the item is more or less a precondition for the diagnosis or it reflects a typical treatment for the patient group. In these instances we omitted the corresponding patient groups from the predictive value calculation. Information on these items and the corresponding subgroups are given in Table 3. The item on HIV was excluded from all analyses, as only one subject outside of the HIV patient group reported a positive test result.

Table 3. Diagnostic value of single items–unadjusted and adjusted for age and gender

Variables*	n	prev	pos	neg	sens	spec	*unOR*	adOR	p
Tonsillectomy	1559	0,36	0,45	0,64	0,44	0,67	*1,84*	1,48	0.0015
Sinus mucosa resection	1559	0,08	0,66	0,63	0,16	0,95	*4,07*	4,63	<0.0001
Appendectomy	1559	0,25	0,46	0,62	0,29	0,77	*2,46*	1,52	0.0034
Splenectomy	1559	0,01	0,52	0,63	0,02	0,99	*5,02*	4,76	0.0045
Abdominal surgery (last 2 years)	1559	0,06	0,63	0,62	0,10	0,95	*3,93*	3,4	<0.0001
Polytrauma	1559	0,05	0,43	0,61	0,05	0,95	*1,36*	1,23	0.4560
Upper resp. tract (URT) infections	1559	0,72	0,42	0,66	0,77	0,31	*1,33*	1,45	0.0084
Lower resp. tract (LRT) infections	1559	0,43	0,52	0,70	0,60	0,68	*3,26*	2,99	<0.0001
Skin infections	1559	0,10	0,62	0,63	0,17	0,94	*3,61*	3,38	<0.0001
Gastrointestinal (GI) infections	1559	0,22	0,53	0,65	0,31	0,84	*2,58*	2,38	<0.0001
Uro-genital tract (UGT) infections	1559	0,13	0,56	0,63	0,18	0,91	*2,58*	2,09	<0.0001
Tooth root infections	1559	0,06	0,55	0,64	0,08	0,96	*3,09*	2,63	0.0001
M.tuberculosis infections	1559	0,03	0,47	0,61	0,04	0,97	*1,83*	1,47	0.2873
Salmonella infections	1559	0,03	0,68	0,62	0,06	0,98	*4,84*	5,55	<0.0001
Chronic viral hepatitis	1559	0,02	0,57	0,61	0,03	0,99	*2,99*	3,86	0.0011
Veneral diseases	1559	0,01	0,62	0,63	0,04	1, 00	*7,27*	10,68	<0.0001
Meningitis	1559	0,02	0,63	0,61	0,03	0,99	*5,29*	6,57	<0.0001
Septic arthritis	1559	0,02	0,40	0,63	0,02	0,98	*1,91*	1,37	0.5196
Septiciemia	1559	0,04	0,60	0,62	0,06	0,98	*3,16*	2,78	0.0011
Tropical diseases	1559	0,02	0,68	0,63	0,04	0,99	*5,17*	4,81	0.0020
Other infections	1559	0,08	0,55	0,62	0,12	0,94	*3,11*	3,07	<0.0001
Inflammatory rheumatitis+	1516	0,17	0,62	0,67	0,27	0,91	*5,37*	3,93	<0.0001
Psoriasis, chronic skin disease	1559	0,08	0,50	0,62	0,12	0,93	*2,24*	2,13	0.0003
Diarrhea, inflammatory bowl disease	1559	0,09	0,77	0,64	0,19	0,97	*8,47*	8,83	<0.0001
Allergic asthma, rhinitis	1559	0,22	0,42	0,62	0,25	0,79	*1,08*	1,09	0.5167
Chronic hematological diseases	1559	0,06	0,86	0,63	0,14	0,98	*11,17*	12,31	<0.0001
Chronic neurological diseases	1559	0,02	0,73	0,61	0,04	0,99	*7,19*	6,12	0.0002
Depression, anxiety	1559	0,10	0,56	0,62	0,14	0,93	*2,87*	2,09	0.0003
Chronic kidney disease	1559	0,03	0,91	0,62	0,06	0,99	*13,24*	11,1	<0.0001
Chronic liver disease	1559	0,02	0,56	0,61	0,04	0,98	*4,46*	4,04	0.0004
Chronic lung disease	1559	0,05	0,86	0,63	0,12	0,99	*15,14*	17,85	<0.0001
Endocarditis	1559	0,01	0,94	0,63	0,02	1,00	*17,30*	20,02	0.0050

Chronic systemic vasculitis+	1516	0,05	1	0,65	0,12	1,00	∞	∞	<0.0000
Diabetes mellitus	1559	0,03	0,49	0,63	0,05	0,97	*2,85*	2,08	0.0480
Coronary heart dis.(CHD), art. hypertention	1559	0,14	0,53	0,61	0,17	0,86	*1,92*	1,55	0.0248
Malignant tumor	1559	0,04	0,50	0,61	0,06	0,97	*2,99*	1,91	0.0690
Antibiotic use	1559	0,07	0,86	0,65	0,18	0,98	*12,62*	15,25	<0.0001
Corticosteriod use	1559	0,16	0,84	0,67	0,31	0,96	*15,08*	11,32	<0.0001
Anti-rheumatic drugs, DMARD+	1516	0,04	0,60	0,65	0,05	0,98	*4,14*	2,47	0.0135
Immunosupressants+	1516	0,08	0,87	0,66	0,17	0,98	*19,71*	15,43	<0.0001
Cytostatic drugs+	1516	0,01	0,92	0,66	0,02	1,00	*23,11*	21,63	0.0034
Immunoglobines iv, sc++	1414	0,01	1	0,70	0,02	1,00	*∞*	∞	<0.0000
Immunostimulating drugs	1559	0,07	0,67	0,63	0,15	0,95	*4,24*	4,17	<0.0001
Childhood vaccination no	1559	0,02	0,61	0,61	0,03	0,98	*2,24*	1,78	0.1936
Childhood vaccinations: no/unknown	1559	0,08	0,44	0,61	0,09	0,92	*1,62*	1,28	0.2769
Vaccination Complications: yes	1559	0,03	0,45	0,61	0,04	0,97	*2,02*	1,12	0.7392
Vacc. Complications: yes or unknown	1559	0,07	0,46	0,61	0,08	0,94	*2,23*	1,59	0.0413
Infections/year >=6	1559	0,08	0,93	0,65	0,20	0,99	*35,84*	38,90	<0.0001
Infections/year >=4	1559	0,21	0,77	0,70	0,44	0,92	*9,90*	10,50	<0.0001
Infections/year >=1	1559	0,81	0,42	0,73	0,88	0,24	*2,20*	2,18	<0.0001
Duration infections > 3w	1559	0,09	0,73	0,63	0,16	0,96	*6,44*	5,25	<0.0001
Duration infections >=3w	1559	0,19	0,72	0,67	0,35	0,91	*8,95*	7,41	<0.0001
Duration infections >=1w	1559	0,70	0,46	0,76	0,84	0,39	*2,77*	2,97	<0.0001
Radiation: yes	1559	0,05	0,31	0,60	0,03	0,94	*0,41*	0,40	0.0022
Radiation: yes or unknown	1559	0,06	0,32	0,60	0,04	0,93	*0,45*	0,42	0.0010

**The number of subjects included, the prevalence of the item, the positive and negative predictive value (prev), sensitivity and specificity, the odds ratio (OR) and the p-value referring to testing the null hypothesis of an OR of 1 are provided. All values are adjusted for age and gender. For comparison the unadjusted OR are given in italic. +=patients of the RA group are excluded from the analysis. ++= patients of the CVID group are excluded from the analysis (for explanation Note that not all items in this list are included in the ISAQ-score calculation)*

To evaluate the value of the ISAQ score, as well as the selected sub-scores, receiver operating characteristic (ROC) curves and corresponding area under the curve (AUC) values were considered. To take the above-mentioned relationship between certain items and patient groups into account, these items did not count in computing the scores for patients from the specific subgroups. The HIV item was not used in computing scores, and neither was the item corticosteroid use, since cortisone is a typical treatment in many patient groups suffering from autoimmunity. To evaluate the value for specific case groups, we also performed analyses comparing the individual case groups with all controls.

For all quantities considered (prevalence, predictive values, sensitivity, specificity, odds ratios and AUCs) we obtained age and gender adjusted values by stratification and averaging over the strata specific results. We used eight strata defined by gender and age with cut points 30, 45, and 60 years, and used weighted averages weighting each stratum with the fraction of subjects of the whole German population in the corresponding group, assuming an overall age range from 18 to 80. If there were strata with no variation of an item such that one predictive value could not be computed, we coarsened the age stratification to two groups based on the cut point 45 years. If this was not sufficient, we adjusted only for age, but not for gender, using two age groups. For the odds ratio we performed these calculations on the log odds scale. For the log odds ratios and the AUC the standard error of the adjusted values were computed by combining the standard errors from each stratum appropriately. If the standard error was undefined in a stratum, we used again coarsened strata. The standard error of the adjusted log odds ratio was used to compute a p-value using the Wald test principle. Confidence intervals for the AUC were computed as +/- 1.96 times the standard error.

To evaluate the potential value of the ISAQ when using an optimal weighting, we used a logistic regression model including all (dichotomized) items and entering the ISAQ-score together with a five-fold cross validation. All computations were performed using Stata 12.1. [8].

3. RESULTS

3.1 Subject Characteristics

Overall 1020 controls and 539 cases were included in our analysis. The size of the different control and case populations, the distribution of gender and age in each population as well as for all controls and cases are shown in Supplementary Table 2. Females constitute 2/3 of all cases , but only 1/3 of the controls. Young patients were overrepresented in the controls (36%) compared to the cases (14%). The substantial difference in age- and gender distribution between cases and controls reflected the different recruitment strategies for the two groups. We took this into account by adjusting all measures considered for age and gender, and found out that this has little impact on the results. Smoking was slightly more prevalent among cases than controls (16.1% versus 13.5%). Body mass index (BMI) distribution in cases and controls was comparable with a tendency of more subjects with BMI <18.5 among cases (7.5%) compared to the controls (3.2%).

3.2 Overall Value of the ISAQ

The distribution of the ISAQ scores in cases and controls (Fig. 1, left) reveals that most control individuals have a score below 35, whereas the majority of cases score 35 or higher.

Actually, the ISAQ median in the cases is 39.5 and that in controls is 30.4. As 93.7% of all controls score lower than the median of the cases, this discrepancy resulted in an AUC of the ROC) curve of 0.838 (95%CI: 0.817-0.859) (Fig. 1, right).

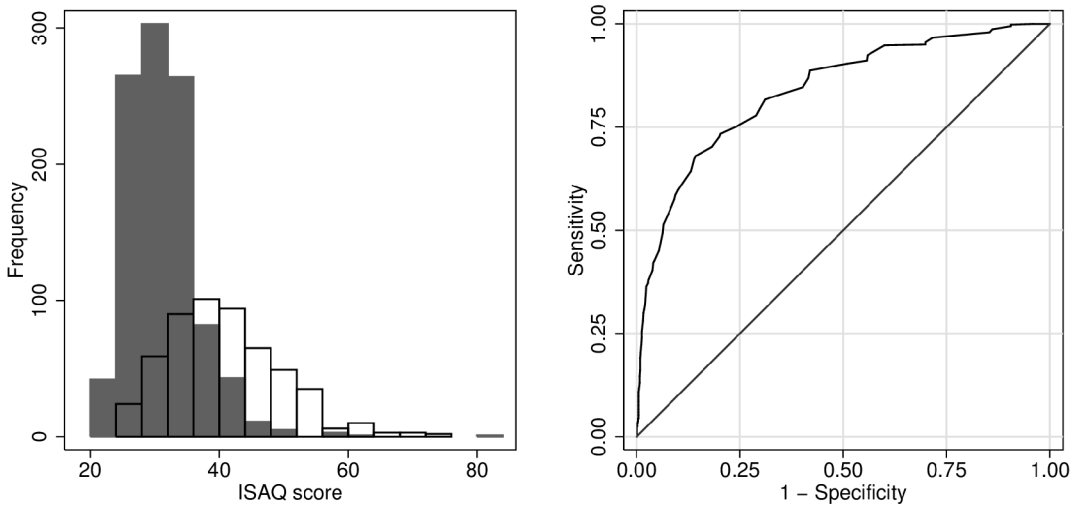

Fig. 1. Left side: Histograms of the distribution of the ISAQ-score in controls (grey columns) and cases (white columns). Right side: ROC curve of the ISAQ-score with an AUC of 0.838 (95%CI: 0.817-0.859)

3.3 Validation of Items

The diagnostic value of each individual item is summarized in Table 3 and illustrated in Fig. 2 as scatter plot of the positive predictive value against the prevalence of each item. There were minor differences between age and gender adjusted and unadjusted values (Table 3, see comparison of adjusted and unadjusted odds ratios).

For some items there was a distinct discrepancy between the original decision to assign the item a low or a high weight and the actual positive predictive value relative to the prevalence. Thus chronic kidney disease, antibiotic use, diarrhoea, and inflammatory rheumatic disease got originally a low weight although they turned out to be among the items with a high predictive value compared to items with similar prevalence. Similarly, duration of infections of 3 weeks seems to have already a similar value than duration of more than 3 weeks. Items with much lower predictive value than anticipated were splenectomy, M. tuberculosis infection, chronic hepatitis, vaccination complications, septic arthritis and in particular, radiation therapy. The latter was actually more frequently reported by controls compared to cases. Fig. 2 illustrates the general difficulty to find items with a high positive predictive value and a relevant prevalence. From this perspective, chronic systemic vasculitis, a frequency of 4 or more infections per year, duration of infections of 3 or more weeks, inflammatory rheumatic disease, gastrointestinal and lower respiratory tract infections are the most promising candidates.

Since for several items the a priori weighting in the ISAQ-score seems suboptimal, an optimal weighting based on a logistic regression approach was investigated. This led to an increase in the AUC of 0.06.

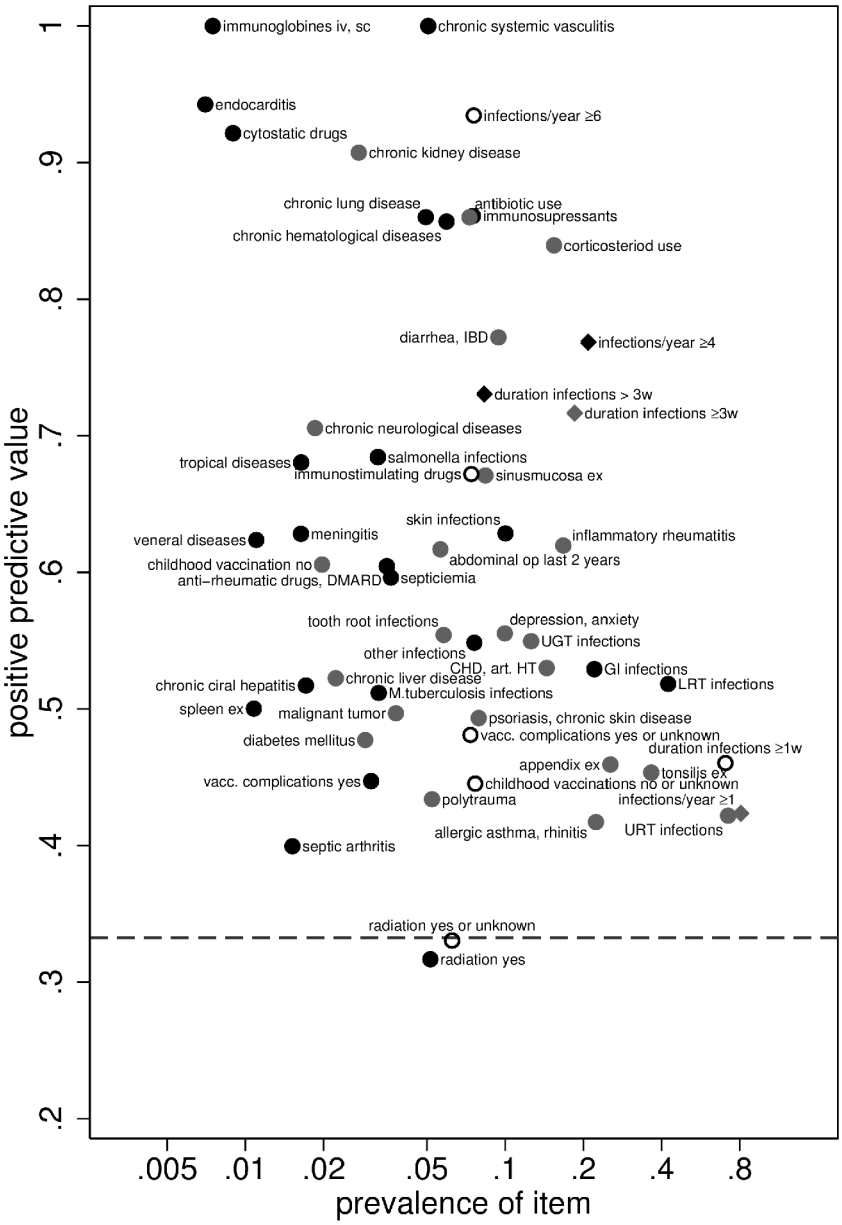

Fig. 2. Scatter plot of positive predictive value versus prevalence for all items considered (adjusted for age and gender). Filled black dot: Binary item included in the ISAQ-score with a weight of 4. Filled gray dot: Binary item included in the ISAQ-score with a weight of 2. Black diamond: Highest category of a trichotomous item included in the ISAQ-score. Grey diamond: Combined highest or second highest category of a trichotomous item included in the ISAQ-score. Hollow dot: Item not included in the ISAQ-score or subcategory not included. The dashed horizontal line corresponds to the prevalence of disease in our population. Note that the prevalence is shown on a logarithmic scale

Table 4. AUC comparison of cases and controls

Group	nCase	nControl	AUC*	low	up	AUCadj	lowadj	upadj
CVID*	145	1020	0,91	0,89	0,93	0,90	0,86	0,93
Cvid1a	46	1020	0,93	0,90	0,96	0,91	0,86	0,96
Cvid1b	46	1020	0,88	0,84	0,93	0,89	0,85	0,93
Cvid2	13	1020	0,93	0,87	0,99	0,93	0,89	0,98
Cvidnd	17	1020	0,93	0,85	1,00	0,93	0,87	0,99
C/ScD*	33	1020	0,83	0,75	0,92	0,85	0,78	0,92
DuID*	80	1020	0,83	0,79	0,88	0,81	0,76	0,87
Immunodeficiency (all)	258	1020	0,88	0,85	0,90	0,86	0,83	0,89
HIV	64	1020	0,72	0,66	0,78	0,67	0,59	0,74
Rheumatoid arthritis	43	1020	0,65	0,57	0,74	0,65	0,55	0,74
Sjoegren syndrome	87	1020	0,84	0,79	0,89	0,82	0,75	0,88
Systemic lupus eryth.	45	1020	0,87	0,81	0,92	0,87	0,82	0,91
Systemic vasculitides	42	1020	0,94	0,91	0,97	0,91	0,85	0,97
Autoimmune disease (all)	217	1020	0,83	0,80	0,86	0,77	0,72	0,83
Age <=30	76	363	0,83	0,77	0,89	0,83	0,77	0,89
Age 31 to 45	193	309	0,84	0,80	0,87	0,83	0,79	0,87
Age 46 to 60	163	234	0,81	0,76	0,85	0,78	0,73	0,83
Age >60	107	114	0,84	0,79	0,89	0,81	0,75	0,88
Female	345	386	0,82	0,79	0,85	0,78	0,74	0,82
Male	184	634	0,84	0,81	0,87	0,84	0,81	0,88
All	539	1020	0,84	0,82	0,86	0,81	0,78	0,84

** AUC (area under the curve) values are indicated when using the ISAQ score to distinguish specific patient groups from controls. The unadjusted AUC as well as the age and gender adjusted AUC (AUC adj) values are shown to together with their 95% confidence intervals (up, low). n(case), n(controls) represent the number of cases and controls used for the specific analysis. For definition of CVID subgroups see 'Materials and Methods'. C/ScD, class and subclass deficiencies. DuID, diverse, unclassified immunodeficiencies*

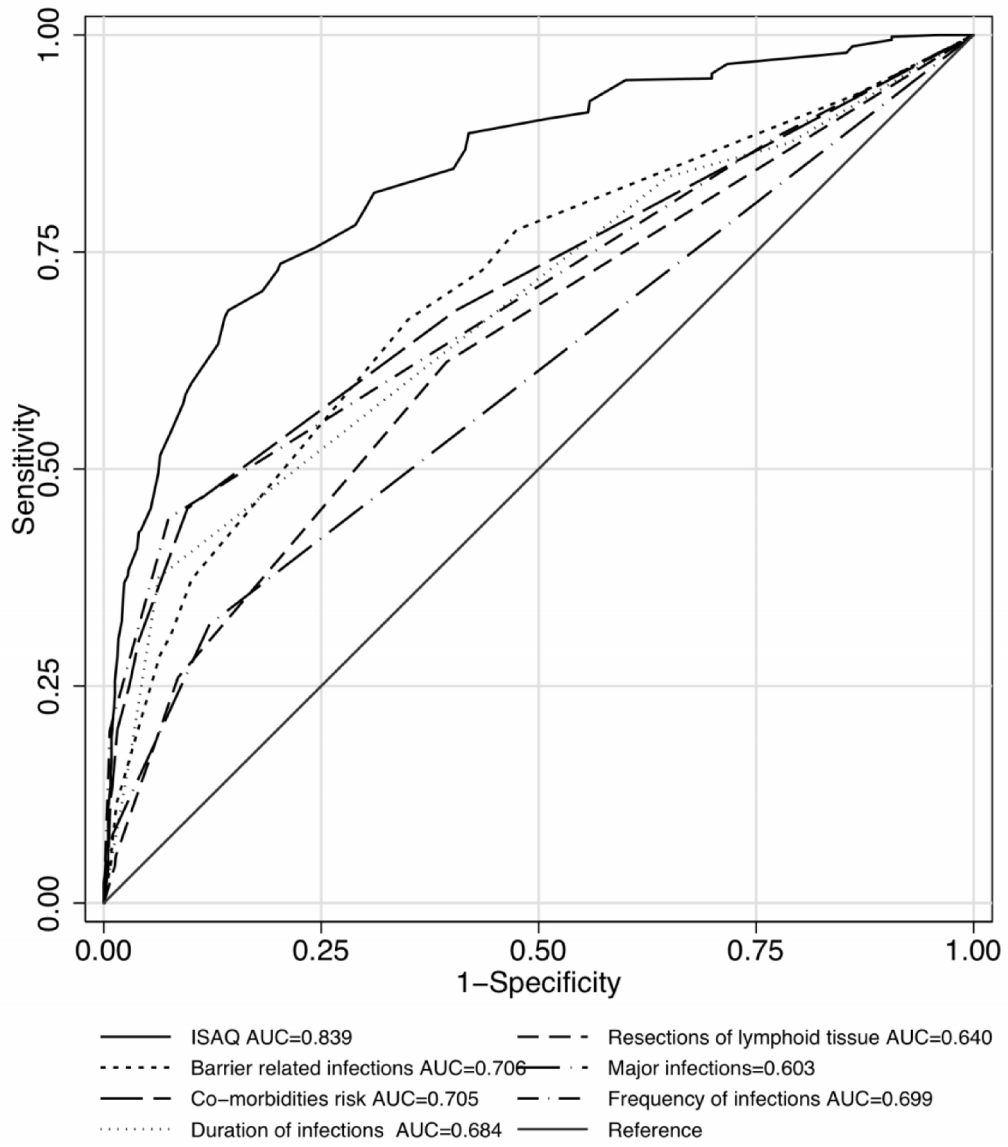

Fig. 3. ROC curves and AUC values for four subscores based on ISAQ topics #5, #12, #14, #15 and two single items addressed in ISAQ questions 11a and b (for details see Table 1). Topic #5: Resection of lymphoid tissue (4 items: resection of tonsils, sinus mucosa, appendix, spleen)

Question 11a: How often do you have infections? (Frequency of infections) (1 item, but ordinally scaled). Question 11b: How long are your infections? (Duration of infections) (1 item, ordinally scaled). Topic #12: Barrier-related infections (6 items: upper respiratory tract, lower respiratory tract, skin gastro-intestinal tract, urogenital tract, tooth roots) .Topic #14: Major ('indicator') infections: (9 items: tuberculosis, sepsis, veneral disease, salmonellosis, meningitis, chronic viral hepatitis, septic arthritis, tropical disease, a.o) .Topic #15: Co-morbidities / general risk (15 items: from rheumatic disease to malignant tumors)

3.4 The Value of ISAQ for Specific Patient Groups

In further analyses the entire control group was compared with eight major patient subgroups as well as the two larger categories of autoimmune diseases (SLE/RA/Sjoegren/vasculitides) and immunodeficiencies (CVID, C/ScD, DuID) seperately. The ISAQ score performed best for CVID and chronic vasculitis patients, and worst for rheumatic diseases and HIV patients. The score seems to better characterize individuals with immunodeficiencies than with autoimmune diseases. Within the clinically best-characterised group of 122 CVID patients there was a trend towards a better discrimination in the most severe CVID subset Ia (Table 4 above). However, when stratifying by age or gender no substantial differences in the AUC values could be observed (lower part of Table 4).

3.5 The Value of ISAQ Subscores

As mentioned in section 3.1., the ISAQ consists of different parts, which allow to define sub-scores for the topics #5 (injuries to the immune system), #12 (localisation of infections), #14 (indicator infections), and #15 (co-morbidities) of the ISAQ (see Table 1). Moreover, some of the single items provide quantitative information, in particular the questions on the frequency and duration of infections (topic 11). In Fig. 3(above) we assess the value of the four different sub-scores and these two single items of the ISAQ. All sub-scores perform distinctly worse than the overall score, but with very similar AUC values in the magnitude of 0.65 to 0.70. On the other side, both single items on the frequency and duration of infections obtain already an AUC of nearly 0.7.

4. DISCUSSION

To our knowledge the ISAQ study is the first attempt to translate modern immunological knowledge and long-standing clinical experience in immune-based diseases into a questionnaire for self-reporting of signs, symptoms, anamnestic features and lifestyle factors. The underlying hypothesis was to construct with this information a score that would be able to identify individuals at risk for immune-based diseases, preferentially immunodeficiency. The selected items have not been validated previously, but were solely selected on the basis of long-standing experience in clinical immunology and rheumatology of two co-authors (HHP, SG). Cohorts of established systemic autoimmune disease and immunodeficiency syndromes served as internal disease controls. The latter ones were grouped into patients with hypogammaglobulinemia of at least two isotypes (e.g. agammaglobulinemia, CVID) requiring intravenous immunoglobulin (IVIg) substitution, Ig class or subclass deficiency (C/ScD) without need of IVig substitution and diverse, unclassified cases (DuID) with increased susceptibility of infections, normal serum Ig levels and no IVIg treatment. Our investigation revealed that the ISAQ-score is to some degree able to discriminate between healthy subjects and patients suffering from chronic immunodeficiency and/or autoimmune diseases. Healthy subjects tend to have rather rarely high values of the ISAQ-score (and some of them may actually suffer from undiagnosed immune-based diseases) whereas in patients with diagnosed disease a substantial fraction scored rather low, suggesting the ISAQ has a high specificity, but only a moderate sensitivity to detect immune dysfunction. Taken into consideration the enormous complexity of the immune system with its multiple interactions, back up loops and memory functions involving barrier defence mechanisms as well as innate and adaptive immune compartments, a low sensitivity of the ISAQ was to be expected. Thus, a patient with impaired specific antibody

formation (such as in CVID) may still be sufficiently protected by proper barrier and innate immune functions as long as the individual is not repeatedly exposed to a high load of virulent pathogens. Conversely impaired barrier and innate immune functions may go along with normal antibody levels not preventing proneness to infections in the "diverse unclassified immunodeficiencies" (DuID) cohort with normal serum Ig levels and in the ANCA associated "chronic vasculitides" with their highly vulnerable mucous membranes and an ANCA-activated neutrophile compartment. The relative high specificity of the ISAQ for immune-based diseases in general might be explained with the remarkable contribution to the total score of some single items such as frequency and duration of infections and antibiotic use. On the other side an unexpected poor predictive contribution was seen for previous injuries to the immune system such as radiation therapy, appendectomy, tonsillectomy and splenectomy, not justifying an increased weighting factor. A possible reason for this may be due to the fact that our question on "radiotherapy" *("Wurden Sie in den letzten beiden Jahren bestrahlt?"* "Did you receive irradiation during the last two years?") did not specify ionising radiotherapy and therefore might have been misinterpreted for any type of irradiation. A similar misinterpretation of the question on resection of lymphoid tissue is less likely, therefore the low predictive value of previous appendectomy, tonsillectomy and splenectomy is probably real whereas surgical resection of sinus mucosa gave a good predictive value (Fig.2). Single items with unexpectedly high predictive values were chronic disease of kidney, lungs, hematopoietic system and inflammatory bowl disease without, however, reaching that of chronic systemic vasculitis (Fig. 2, Table 3). Taken together the single item analysis allows us to rank the various questions with respect to their predictive value and diagnostic strength.

Although the ISAQ performs rather uniformly over many different subjects, age and gender groups it seems to be better targeted for immunodeficiency syndromes (notably CVID) when compared to autoimmune diseases such as SLE, RA and Sjögren's. This tendency becomes even more relevant when keeping in mind the high single item contribution of immunosuppressive drugs, which are usually given in autoimmune diseases and not in immunodeficient patients.

We could not find evidence for any subsection of the ISAQ alone being able to substitute for the whole IASQ. However, some single items of the ISAQ, in particular those on frequency and duration of infections, antibiotic use and the above mentioned chronic renal, pulmonary and haematological co-morbidities, provide already alone valuable information. This may suggest the application of a short form of the ISAQ with a few questions if the full ISAQ cannot be applied due to limited resources.

A basic limitation of the study is the retrospective nature of our data and the lack of a blinded cohort of patients. The ISAQ was applied in patients with a known diagnosis, and the response of the patients to some items might have been different prior to the diagnosis. We tried to take this into account by different weighting factors and by not counting some items in some patient groups, where the item reflected either a precondition or a typical treatment (e.g. IVIg therapy in CVID, immunosuppressive treatment in RA). In any case, the ISAQ requires further validation, ideally in prospective studies, where subjects with a high risk according to the ISAQ are clinically examined and followed up for several years for immune-based diseases and incident infections.

A further limitation of our study is the substantial difference in age- and gender distribution between cases and controls, reflecting the different recruitment strategies for the two groups.

We took this into account by adjusting all measures considered for age and gender, and actually we found out that this has little impact on the results.

The ISAQ in its present form includes several items of limited value. Moreover, the weighting of items seems to be suboptimal and the formulation of some questions needs to be more succinct. A logistic regression model using all items suggests potential for optimizing the weighting factors. The construction and validation of an optimized version of the ISAQ and its scoring logic should be part of further prospective studies or of retrospective studies with careful selection of cases and population-based controls. To the best of our knowledge a similar validation of questions related to the functioning of the immune system have not been undertaken in previous studies. In the field of rheumatic diseases self-reporting questionnaires have been proposed and validated [9,10]. Moreover, recently the RABBIT risk score for serious infections applicable in RA prior to the switch from conventional disease modifying anti-rheumatic drugs (DMARDs) to anti-TNFa blocking agents has been validated and proven to be useful [11,12]. However, this score included information on previous and actual treatment and is hence not a self-reporting, diagnostic tool for a general assessment of the immune system. An improved ISAQ score, on the other hand, may serve as a valuable tool to stratify patients in clinical studies involving immunomodulatory or immunosuppressive treatments.

It remains an open question why the ISAQ failed to identify a substantial fraction of the cases. This may reflect subgroups of patients with no observable signs (e.g. remission), other signs than covered by the ISAQ or patients unable or unwilling to respond to items of the ISAQ. Also this should be a topic of future research.

5. CONCLUSION

A novel self-reporting questionnaire (ISAQ) was designed to assess individuals for the functionality of their immune system. The ISAQ derived score was probed in healthy adults and in patients with various forms immunodeficiency and chronic autoimmune disease. A marked difference of the ISAQ scores between cases and controls was evident, suggesting that this tool has potential to identify individuals at risk for immune-based diseases, particularly with immunodeficiency phenotypes or systemic vasculitis. However, as items with high and low predictive power were identified, the ISAQ in its present form is suboptimal and requires refinement preferentially in the context of longitudinal studies.

CONSENT

All patients and controls were orally informed about the scientific purpose of the ISAQ study and about their free decision to take part or not in the study. They were instructed about the anonymous character of the study allowing via an individual 6-digit ID code only the study participant and the study team to access the calculated ISAQ score. All patients and controls gave oral consent to the study principles and were freely filling out the ISAQ form.

ETHICAL APPROVAL

All authors hereby declare that the Ethics Committee of Freiburg University Medical Centre (UMC) approved the ISAQ project (Project Nr. 174/08) and that it was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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