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Title	The Immune Status Questionnaire (ISQ): A critical review
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Published in	Journal of Clinical and Basic Psychosomatics
Publication Date	2025-06-17
Link	https://dspace.library.uu.nl/handle/1874/480375
Citation	Išerić, E, Garssen, J & Verster, J 2025, 'The Immune Status Questionnaire (ISQ) : A critical review', Journal of Clinical and Basic Psychosomatics, vol. 4, no. 1, 025150030. https://doi.org/10.36922/JCBP025150030
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ACCSCIENCE PUBLISHING

Journal of Clinical and Basic Psychosomatics

REVIEW ARTICLE

The immune status questionnaire: A critical review

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Abstract

Immune fitness, defined as the adequate functioning of the immune system, is essential for maintaining health, preventing and resolving disease, and improving quality of life. The immune status questionnaire (ISQ) is a self-assessment tool developed to evaluate the type and frequency of seven common immune-related complaints. This review was conducted to provide a comprehensive overview of studies that utilized the ISQ and provide recommendations for its future improvement. A literature search on Google Scholar, supplemented by cross-referencing, identified 91 publications, of which 51 (from 38 independent studies) reported ISQ data and were included in this review. These studies were conducted in 14 countries, with the ISQ translated into six languages. They were cross-sectional and experimental studies involving healthy subjects or patient populations, with sample sizes ranging from 22 to 45,782 participants. Findings revealed that ISQ scores significantly differed across demographics (e.g., sex and age) and health status (e.g., obesity and diabetes). Significant correlations were found between ISQ scores and various health correlates, including positive correlations with lifestyle factors – such as regular physical activity, attaining a daily diet, and adequate sleep – and negative correlations with mood states such as anxiety and depression, alcohol use, hangovers, and smoking. In addition, the ISQ scores also showed associations with immune biomarkers indicative of systemic inflammation. The review also discusses the strengths and limitations of the ISQ, including its 12-month recall period, which may be adapted in repeated-measures designs. Potential improvements to the ISQ include incorporating additional characteristics such as duration, severity, and impact of immune-related complaints and refining the current scoring method. In conclusion, the ISQ is a simple, cost- and time-effective questionnaire to assess immune fitness. The ISQ can be successfully implemented in research and screening in clinical settings.

Keywords: Immune fitness; Immune status questionnaire; Perceived immunity; Systemic inflammation

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Citation: Išerić E, Garssen J, Verster JC. The immune status questionnaire: A critical review. *J Clin Basic Psychosom.* 2026;4(1):1-17.
doi: 10.36922/JCBP025150030

Received: April 9, 2025

1st revised: April 28, 2025

2nd revised: May 19, 2025

Accepted: May 28, 2025

Published online: June 17, 2025

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1. Introduction

Immunity is a vital determinant of health and survival.[1] Without a proper immune response, the body cannot defend against various ranges of external pathogens, including bacteria, viruses, fungi, and parasites, as well as environmental toxins.[1] This lack of protection can lead to life-threatening infections.[2] The immune system is also responsible for recognizing disease-inducing changes that occur internally, such as the development of cancer cells.[1] If these changes are not detected by the immune system, they can result in systemic inflammation and the development of immune-related diseases.[2] Conversely, if the immune system becomes overactive and targets healthy cells, this can lead to autoimmune diseases.[2]

Immune fitness refers to the body's capacity to respond to health challenges (such as infections), by activating an appropriate immune response. This is essential to maintain health, prevent, and resolve disease and ultimately maintain or improve quality of life.[3,4] Preventing systemic inflammation is important, as it has been linked to 9 out of 10 most frequently reported causes of death in the United States,[5] including heart disease, cancer, the 2019 coronavirus disease (COVID-19), cardiovascular disease, chronic lower respiratory diseases, Alzheimer's disease, diabetes, chronic liver disease and cirrhosis, and kidney disease.[6-9]

It is estimated that immune-related non-communicable diseases (NCDs) impact approximately 10% of the global population, with the incidence rate continuing to rise worldwide.[5,7-9] According to data from the Global Burden of Diseases Study,[5] the global incidence of six major immune-related NCDs was 67,586,168 cases in 2019. Systemic inflammation can also result from communicable diseases (e.g., viral infections),[10,11] and many patient-reported health complaints in clinical practice (e.g., common cold, headache) are immune-related.[12] The burden of immune-related diseases and complaints highlights the importance of early detection of systemic inflammation and the assessment of immune fitness per se.

Conventionally, immune status is assessed through biomarkers of systemic inflammation in biological matrices such as blood, stool, and saliva.[3,4] These inflammatory markers include C-reactive protein (CRP), fibrinogen, and various cytokines, such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ.[13,14] Biomarker assessments are usually conducted after consulting a healthcare professional when signs or symptoms of immune-related complaints or diseases are present. It can then be determined whether biomarker concentrations are within the normal range.

Patients are usually unaware of changes in biomarker levels, and objective changes do not always correspond to subjective health perceptions. For example, while inflammation may be present in cases of immune-related complaints, such complaints can also be experienced without any measurable changes in biomarker concentrations.[3,4] Conversely, similar to medical conditions such as hypertension, biomarkers can be out of range even though the patient remains asymptomatic. As a result, correlations between biomarkers of systemic inflammation and immune fitness are often modest.[14] These discrepancies are of concern, as a patient's perception of immune functioning will ultimately determine whether or not medical help is sought (e.g., visiting a physician), and whether behavioral or lifestyle changes are initiated (e.g., quitting smoking). It is therefore critical to assess patient perception of immune status, for example through interviews or questionnaires. Taken together, there is a clear need for alternative assessment tools of immune status, either alone or in conjunction with biomarker assessments. In this context, the immune status questionnaire (ISQ) was developed[15] (Figure 1).

The ISQ was derived from a broader list of 23 immune-related complaints, and regression analysis was conducted to identify the seven primary items that predicted 85% of overall immune fitness (i.e., the sum score of the items).[15] The ISQ is a Likert-type scale that assesses the type and frequency of seven common immune-related complaints, including sudden high fever, diarrhea, headache, skin problems (e.g., acne and eczema), muscle and joint pain, common cold, and coughing (Figure 1). Respondents indicate the frequency of each complaint experienced in the past year using a 5-point Likert scale ranging from 0 (never), 1 (sometimes), 2 (regularly), 3 (often), to 4 (almost always). The sum score of the seven items ranges from 0 to 35 and is converted to a final ISQ score ranging from 0 (very poor) to 10 (excellent) (Figure 2).

The seven validation studies by Wilod Versprille *et al.*,[15] conducted in Dutch or English, revealed that ISQ

Immune Status Questionnaire (ISQ)

Please indicate how often you have had the following complaints **in the past 12 months**:

	Never	Sometimes	Regularly	Often	(Almost) always
Sudden high fever	○	○	○	○	○
Diarrhea	○	○	○	○	○
Headache	○	○	○	○	○
Skin problems (e.g. acne & eczema)	○	○	○	○	○
Muscle and joint pain	○	○	○	○	○
Common Cold	○	○	○	○	○
Coughing	○	○	○	○	○

Figure 1. A sample of the immune status questionnaire

Item sum score	≥15	14	13	12,11	10	9,8	7	6	5	4,3	≤2
Recoded score	0	1	2	3	4	5	6	7	8	9	10

Figure 2. Recoding the sum score of the seven immune status questionnaire items

scores could differentiate between health and disease and correlate significantly with disease severity and mood. The ISQ scores also correlated significantly with a single-item assessment of momentary immune fitness. The validation studies revealed adequate test-retest reliability (r = 0.80). However, since the seven items can occur independently and represent different immune-related complaints, the internal consistency of the ISQ is only modest (Cronbach's alpha = 0.471 – 0.632). This is further reflected by the low inter-item correlations (r < 0.2). Up to now, no attempts have been made to identify a possible underlying factor structure of the ISQ, which could provide more information about the inter-relationship between the items. Finally, a cut-off score of <6 was proposed to indicate reduced immune fitness.

The aim of this review is to provide a complete overview of studies that have utilized the ISQ in research and clinical practice, including validation in various languages. The review discusses the extent to which the ISQ outcomes relate to health and lifestyle factors and immune biomarker assessments. In addition, recommendations are made regarding the use of the ISQ and potential improvements to the current version.

2. Methods

To identify potential studies for this systemic review, a search was conducted on Google Scholar on 19 February 2025 for all publications cited in the ISQ validation study by Wilod Versprille *et al.*[15] The search retrieved 87 publications. Cross-referencing yielded an additional five publications. In total, 92 publications were considered. Of these, 51 publications were included because the ISQ was used in their research, and/or data on the ISQ were presented. The remaining 41 publications were excluded for the following reasons: duplicates (e.g., the same publication or an abstract later published as a full article; $n = 6$), written in Persian or Russian ($n = 2$), the ISQ was not assessed (e.g., the scale was mentioned but no new data was presented; $n = 30$), the publication was not peer-reviewed (e.g., an undergraduate thesis; $n = 2$), or the publication could not be retrieved ($n = 1$).

3. Results

A total of 51 publications were included. In some cases, multiple publications originated from the same study. Overall, data were collected from 37 studies conducted across 14 countries. Most studies originated from Indonesia ($n = 9$) and the Netherlands ($n = 7$), followed by Saudi Arabia ($n = 5$), India ($n = 4$), and the United States ($n = 3$). Other countries represented by one study each included Germany, Greece, Fiji, Italy, Ireland, Jordan, Pakistan, New Zealand, and Malaysia. Five publications presented pooled data analyses incorporating data from multiple countries. The ISQ has been translated into six languages. Most often, the ISQ was administered in English, Dutch, or Bahasa Indonesian. Other studies used unvalidated ISQ versions in Italian, Arabic, and Malay. The sample size ranged from 22 to 45,782 participants, and sample characteristics varied depending on the study objectives. All studies recruited participants aged 18 years and older. The four main sample groups were the general population, student populations, personnel from companies or institutions, and individuals with a diagnosed condition or complaint. Due to the retrospective nature of the ISQ, most of the studies were cross-sectional ($n = 42$), assessing ISQ data at a single time point. Two studies validated the ISQ in the Indonesian language, with one including a test–retest analysis. In addition, seven experimental studies examined ISQ scores changes before and after an intervention.

3.1. Validation studies in other languages

Two validation studies translated the ISQ into Bahasa Indonesian language. Maulana and Arovah[16] tested the psychometric properties of the Indonesian ISQ in 296 adults. They found that the ISQ demonstrated sufficient validity (significant correlations with health-related quality of life assessed using the SF-12), adequate internal consistency (Cronbach's alpha = 0.70 – 0.88), and good test–retest reliability (r = 0.87 – 0.82). In a second, smaller validation study, Azhar *et al.*[17] tested the Bahasa Indonesian ISQ among 30 patients who visited Hasan Sadikin General Hospital for a general health checkup. They concluded that the translated ISQ is a reliable instrument (Cronbach's alpha = 0.706). However, this study did not include a test–retest assessment, and validity was inferred from significant correlations between each individual item and the total ISQ score. The ISQ has also been translated and used in Arabic and Italian. However, no formal validation studies have been published for these language versions.

3.2. Cross-sectional studies relating the ISQ to health and lifestyle correlates

Thirty-seven publications reported survey studies investigating the relationship between ISQ scores and demographic characteristics, health outcomes, disease

states, and lifestyle factors. Abdulahad *et al.*[18] examined irritable bowel syndrome (IBS) complaints among 871 Dutch adults with self-reported insomnia. The online survey, conducted in Dutch, used an earlier ISQ scoring format ranging from 0 (excellent) to 28 (very poor). The presence and severity of IBS symptoms were evaluated using a modified Dutch version of the Birmingham IBS Symptom Questionnaire.[19,20] Poorer ISQ scores were significantly correlated with higher total IBS scores as well as increased reports of constipation, abdominal pain, and diarrhea. Figure 3 shows these correlations after recoding the ISQ scores into the current 0 – 10 scoring format. Poorer immune fitness also correlated significantly with having more frequent nightly awakenings and poorer sleep quality.

Baars *et al.*[21] assessed whether the consumption of fermented milk products was associated with improved immune fitness. A retrospective consumer survey was conducted in Dutch among 390 adults who bought fermented milk products. This early study also used the 0 – 28 ISQ scoring system. The participants reported a significant improvement in immune fitness after beginning consumption of raw fermented milk products. This improvement was observed in both a poor-health group and a normal-health group and was more pronounced in females than males.

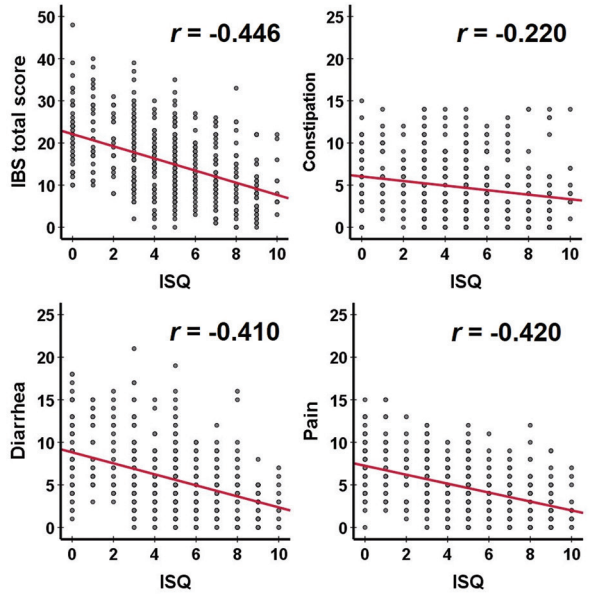

Figure 3. Correlations between the ISQ scores and IBS-related complaints. All Spearman's correlations were statistically significant (p<0.05). Data from Abdulahad *et al.*[18]
Abbreviations: IBS: Irritable bowel syndrome; ISQ: Immune status questionnaire.

Hendriksen *et al.*[22,23] conducted a study among 341 pharmacy students, PhD candidates, and postdoctoral researchers from Utrecht University, the Netherlands, to retrospectively assess the impact of the COVID-19 pandemic on mood and academic performance. An online survey was administered in English or Dutch. Pre-pandemic ISQ scores showed no significant correlations with changes in mood or academic performance during the pandemic – assessed as the average of two lockdown periods. However, further analysis of the 2019 pre-pandemic data[23] revealed that ISQ scores correlated significantly and negatively with stress (r = −0.212, p<0.001), anxiety (r = −0.265, p<0.001), depression (r = −0.204, p<0.001), fatigue (r = −0.244, p<0.001), and loneliness (r = −0.177, p=0.004). ISQ scores were positively correlated with sleep quality (r = 0.210, p<0.001) but showed no significant correlations with optimism, happiness, and quality of life. Using the same dataset, Balikji *et al.*[24] compared participants who reported impaired wound healing (i.e., slow-healing wounds and/or wound infections) with healthy controls. ISQ scores were significantly lower in the impaired wound healing group compared to the control group, particularly on the items assessing diarrhea, headache, skin problems, coughing, and muscle and joint pain.

The study by Hendriksen *et al.*[22,23] was replicated in Germany by Koyun *et al.*,[25] involving 317 young adults (18 – 35 years old) who completed the survey in English. Further analysis of the 2019 pre-pandemic data revealed that ISQ scores correlated significantly and negatively with stress (r = −0.161, p=0.006), anxiety (r = −0.214, p<0.001), depression (r = −0.156, p=0.008), fatigue (r = −0.230, p<0.001), and loneliness (r = −0.144, p=0.015). No significant correlations were found between ISQ scores and optimism, happiness, quality of life, and sleep quality.

Kiani *et al.*[26] examined whether immune fitness in 2019, as measured by the ISQ, could predict the presence and severity of COVID-19 symptoms during the pandemic in the Netherlands. Data were drawn from two studies: the "Corona: How Fit Are You?" study (CLOFIT; n = 1415; Dutch adults)[27] and the Corona Test Street Study (COTEST; n = 88; Dutch adults who tested positive for severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]).[28] Alongside immune fitness, various sociodemographic, health, and lifestyle factors were included in regression analyses. For the CLOFIT dataset, ISQ scores before the pandemic were the strongest predictors of both the number and severity of COVID-19 symptoms (explaining 20.1% and 19.8% of the variance, respectively). In the COTEST dataset, pre-pandemic ISQ scores were the only significant predictor of both symptom number (27.2%) and severity (33.1%).

Sips *et al.*[29] examined the impact of reduced immune fitness on work performance. Using data from 425 working adults from the CLOFIT sample,[27] the estimated economic cost of reduced immune fitness for the Dutch economy in 2019 was €10.7 billion. This estimate was based on the average number of absenteeism days (2.9 days per person) and reduced performance (−22.8%) on presenteeism days (19 days per person). The ISQ scores showed a significant negative correlation with the number of absenteeism and presenteeism days as well as with performance levels on presenteeism days. In another CLOFIT subsample ($n = 505$), Hendriksen *et al.*[30] found no significant differences in pre-COVID-19 ISQ scores between participants living alone and those living with family or friends.

Verster *et al.*[4] further examined data from the CLOFIT study to compare the ISQ scores between individuals with and without underlying disease. Participants (n = 1400) reported whether they had any of the following conditions: cardiovascular disease or hypertension, diabetes, liver disease, neurological disease, immune disorders, allergies, kidney disease, pulmonary disease, anxiety, depression, sleep disorders, or other chronic diseases. Of these, 485 reported no underlying conditions, while others reported one (n = 484), two (n = 253), three (n = 108), four (n = 42), or five or more (n = 28). ISQ scores were significantly lower among those with underlying diseases, with scores decreasing further as the number of reported conditions increased (Figure 4).

Other publications confirmed the relationship between ISQ scores and disease state. A comparison between 100 diabetes patients with 100 sex- and age-matched healthy controls from the CLOFIT study revealed that individuals

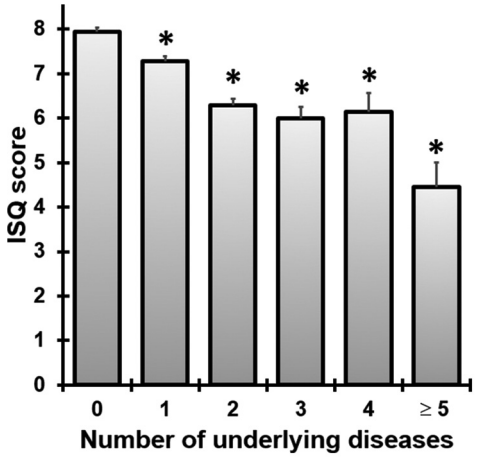

Figure 4. ISQ scores of individuals with and without underlying diseases. Mean and standard errors are shown.
*Indicates significant differences ($p<0.05$).
Abbreviation: ISQ: Immune status questionnaire.

with diabetes had significantly lower ISQ scores.[31] In addition, analysis of 1020 pre-pandemic ISQ scores from CLOFIT showed that 2019 ISQ scores significantly and negatively correlated with both the frequency of common cold episodes in the first 2.5 months of 2020 ($r = -0.212$, $p<0.001$) and the average severity of common cold complaints, as measured by the Jackson Cold Scale ($r = -0.231$, $p<0.001$).[32]

In Greece, Verster *et al.*[33] surveyed 299 international tourists before and after walking the 15.8 km Samaria Gorge. The surveys were completed in English during the bus journey to and from the trail. The data revealed that ISQ scores were a significant predictor of the duration of the walk, with better immune fitness associated with a shorter walking duration and lower water consumption during the hike. However, ISQ scores did not predict exhaustion level, number and duration of breaks, or perceived effort needed to complete the walk. Participants who consume alcohol reported significantly lower ISQ scores compared to non-drinkers.

Tarantino *et al.*[34] examined the impact of perceived stress and immune status on decision-making during the first COVID-19 pandemic lockdown in Italy. A total of 250 adults (aged 19 – 69) completed the ISQ (in Italian), along with the Iowa Gambling Task and a Go/No-Go task. A different ISQ scoring system was used, where higher scores indicated poorer immune status. Younger participants (<34 years old) reported significantly poorer immune fitness than older participants. In addition, females reported poorer immune fitness than males. Poorer immune fitness correlated significantly with greater impulsivity (i.e., disadvantageous decision-making on the gambling tasks) in males but not in females. Adequate immune fitness in younger males was associated with making advantageous choices, whereas poorer immune fitness was associated with making risky, disadvantageous choices in older males.

Kiani *et al.*[35] conducted a pooled analysis of data from 12 studies across various countries to examine the relationship between immune fitness and body mass index (BMI). Data from 4263 adults were included. Compared to individuals with normal weight, ISQ scores were significantly lower among those who were underweight and classified in obesity class III. Using the same pooled dataset, Mulder *et al.*[36] consistently found across all age groups that females reported significantly lower ISQ scores than males, except in the elderly group (≥65 years). The authors proposed that women may report immune-related complaints more thoroughly, including minor symptoms, which could lead to higher overall scores. Interestingly, ISQ scores increased with age, which contrasts with literature

showing age-related decline in immune function.[37] This discrepancy may be due to sampling bias, as online surveys are more likely to attract healthier elderly individuals, thereby skewing results.

Five cross-sectional studies utilizing the ISQ were conducted in Saudi Arabia.[38-42] In one study involving 252 adults, Alfawaz *et al.*[38] administered the ISQ in Arabic and reported a positive significant correlation between ISQ scores and dietary zinc intake. Another survey among 327 adults found a significant positive correlation between ISQ and adherence to the Mediterranean diet.[39] A survey among 1721 national and foreign adult and elderly residents was conducted in both English and Arabic.[40] The study found a significant negative association between ISQ scores and physiological distress during the COVID-19 pandemic, with the strongest effects observed in elderly people. A significant positive association was also found between ISQ scores and the frequency of vitamin D and vitamin C intake. Alharbi *et al.*[41] surveyed 925 national and foreign adult residents and found that having an ISQ score below 6 was significantly associated with obesity, smoking, and a shorter sleep duration. In a separate study, Alharbi[42] surveyed 221 Saudi medical students in both English and Arabic. ISQ scores were significantly and positively correlated with adherence to an exercise program and significantly and negatively correlated with fatty food consumption, daytime sleepiness, and BMI.

In Jordan, Nawaiseh *et al.*[43] conducted a survey among 615 adults (aged 18 – 65) using the Arabic version of the ISQ. A significant positive association was found between ISQ scores and the frequency of vitamin D and vitamin C intake during the COVID-19 pandemic.

Seven studies involving the ISQ originated in Indonesia.[44-50] Ramadhia *et al.*[44] conducted a survey among 47 employees of the Research and Development Agency for Law and Human Rights (Balitbang Hukum dan HAM). ISQ scores, assessed in Bahasa Indonesia, were significantly and negatively correlated with sleep duration ($r = -0.366$, $p=0.011$) but not significantly correlated with dietary nutrient intake (vitamins A, C, D, E, iron, and zinc) and BMI. Purnama and Anindya[45] examined future anxiety among 102 Indonesian nursing students treating COVID-19 patients. The ISQ and survey were completed in Bahasa Indonesia. Future anxiety was assessed with the Future Anxiety Index. Anxiety scores were relatively high among the participants, while 59.8% reported having good immune fitness. No significant relationship was found between the students' ISQ scores and future anxiety, including psychological, social, environmental, economic, media, religious, and general anxiety. Sudrajat *et al.*[46] evaluated 110 adults from the Cibubur Village Community Health Center on immune fitness and attitudes toward herbal consumption during the pandemic. The ISQ (in Bahasa Indonesia) revealed no significant differences in scores based on sex or education. However, participants with jobs had lower ISQ scores than unemployed individuals. Positive associations were found between ISQ scores and age, as well as knowledge and positive attitudes toward herbs.

Chandla *et al.*[47] surveyed 402 COVID-19-positive patients to examine whether ISQ scores differed between symptomatic and asymptomatic individuals. The ISQ, completed in English, showed no significant differences between the two groups.

Purwanto *et al.*[48] conducted a study among 42 Indonesian teachers who followed a community service course involving lectures and daily exercise instructions. ISQ scores, assessed before and after the course in Bahasa Indonesia, significantly improved from 3.48 to 6.64, along with improvements in immune fitness knowledge. In a related study, Purwanto *et al.*[49] examined 170 Indonesian medicine and health science students and found that males reported better immune fitness than females. In addition, regular sports participation was associated with higher ISQ scores and lower incidences of headaches, skin problems, and muscle and joint pain. Finally, Susmitha and Simamora[50] surveyed 120 Indonesian law students and found that better knowledge of infectious diseases and immune fitness was strongly correlated with higher ISQ scores ($r = 0.782$).

In Pakistan, Kazmi and Iftikhar[51,52] examined biopsychosocial determinants of quality of life in 400 patients with hepatitis B or C. In addition to the ISQ, quality of life, mood, and personality were assessed. Regression analysis identified ISQ scores as significant predictors of quality of life. Significant negative correlations were found between ISQ scores and various dimensions of psychological distress, including depression, anger, mania, anxiety, suicidal ideation, psychosis, sleep and memory issues, repetitive thoughts and behaviors, dissociation, personality functioning, and mental resilience.

In the United States, Huang *et al.*[53] examined physical activity, immune fitness, and quality of life during the COVID-19 pandemic in a sample of 467 adults aged over 20. The survey was conducted through the Amazon Mechanical Turk platform in English and the ISQ was assessed for the past 6 months. Based on their score on the international physical activity questionnaire-short form,[54] participants were categorized into active or sedentary groups. Regression analysis revealed that better immune fitness was a significant predictor of an active lifestyle during the pandemic. However, ISQ scores were not a significant predictor of a sedentary lifestyle.

Billot *et al.*[55] examined the relationship between anxiety and immune fitness among 390 residents of New Zealand, including indigenous Māori participants aged 19 – 88 years. The survey was conducted in English. In both Māori and non-Māori participants, higher anxiety levels were strongly linked to poorer immune fitness as measured by ISQ scores.

Breeze *et al.*[56] combined data from the general population and university students in Italy, New Zealand, and India (n = 2,482) and also found that anxiety was significantly and negatively correlated with ISQ scores. Further network analysis by Chalmers *et al.*[57] confirmed this finding, revealing that anxiety scores were also positively correlated to depression and stress. Among these variables, stress but not depression was directly correlated to poorer immune fitness. While mindfulness, mental resilience, and positive affect were positively associated with quality of life, none of these were direct predictors of immune fitness. Notably, quality of life was positively related to immune fitness in participants from New Zealand and India, but not Italy, suggesting that cultural differences may influence these relationships. In terms of demographics, sex emerged as a relevant factor: females in New Zealand and Italy had significantly lower ISQ scores compared to males, a pattern not observed in India.

Holland *et al.*[58] investigated the relationship between immune fitness and cognitive functioning in long COVID-19. The study was conducted among 71 Irish long COVID-19 patients and 50 healthy controls. Participants completed the ISQ along with questionnaires assessing anxiety, depression, fatigue, and cognitive functioning (i.e., absent-mindedness, slips and errors of perception, memory problems, and psychomotor impairment) using the Cognitive Failures Questionnaire (CFQ).[59] The long COVID-19 patients had significantly poorer immune fitness than the control group (mean ISQ score: 4.36 versus 8.97, p<0.001) and reported more cognitive difficulties, including higher scores on depression and fatigue. Regression analysis revealed that immune fitness significantly predicted cognitive functioning (CFQ score), even after controlling for depression, fatigue, and other risk factors such as BMI, sex, and age. In contrast, a Dutch study by Kiani *et al.*[60] involving 299 long COVID-19 patients found that pre-infection ISQ scores (for the year before SARS-CoV-2 infection) did not significantly predict fatigue, mood, and immune fitness during long COIVID-19.

Verster *et al.*[61] conducted a study among 333 international young adults (aged 18 – 35) visiting Fiji for work or holiday, examining the impact of the change in environment on immune fitness. The survey was conducted in English. ISQ scores significantly improved while participants were in Fiji – regardless of the purpose of visit. The same cohort was also analyzed for alcohol consumption and hangover severity.[62] Results showed that subjective intoxication was the strongest predictor of next-day hangover severity. ISQ scores also significantly contributed to this prediction model, especially among women. However, ISQ scores did not predict subjective intoxication levels or the quantity of alcohol consumed. Further analysis of the Fiji dataset revealed significant correlations between ISQ scores and various mood states experienced at home, including stress (r = −0.183, p<0.001), anxiety (r = −0.145, p=0.008), depression (r = −0.148, p=0.007), fatigue (r = −0.148, p=0.007), loneliness (r = −0.144, p=0.015), and neuroticism (r = −0.250, p<0.001). No significant correlations were found between ISQ scores and hostility.

3.3. Relationship with momentary immune fitness

The assessment of current, momentary immune fitness is usually conducted with a single-item rating scale, ranging from 0 (very poor) to 10 (excellent) (Figure 5). A definition of immune fitness is provided to aid participant understanding. In several studies, past year's immune fitness, as assessed by the ISQ, was compared with current immune fitness.

The validation studies by Wilod Versprille *et al.*[15] reported significant but modest correlations between ISQ scores and current immune fitness (r = ~0.4). Other studies have also investigated the predictive validity of past year's immune fitness (ISQ) for current immune fitness, generally finding modest correlations. In a study by Mulder *et al.*,[14] data from 108 Dutch students revealed a significant correlation between ISQ scores and a momentary single-item immune fitness rating (r = 0.452, p<0.001).[63] Significant correlations were specifically found between current overall immune fitness and the past-year frequency of experiencing fever, headache, and common cold. Other immune-related complaints assessed by the ISQ did not show significant correlations. A pooled analysis from multiple studies,

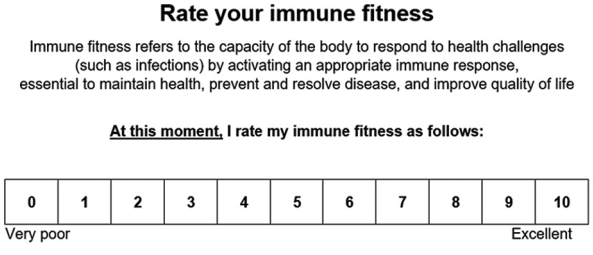

Figure 5. A sample of the single-item assessment for current, momentary immune fitness. Adapted from Verster *et al.*[3]

with a combined sample size of 3,748 participants, revealed a significant and positive correlation between ISQ scores and current immune fitness (r = 0.407, $p<0.001$).[3] Another pooled analysis involving 4,272 participants showed a significant positive correlation between the ISQ score and a single-item assessment of general health (r = 0.593, $p<0.001$).[3]

3.4. Relationship between ISQ scores and immune biomarker assessments

To date, only a few studies have investigated both immune biomarkers and ISQ scores. Mulder *et al.*[14] tested 108 Dutch students aged 18 – 30 years, who completed several questionnaires on mood and health, including the ISQ. Momentary immune fitness was assessed using a single-item rating scale ranging from 0 (very poor) to 10 (excellent) (Figure 5). Participants provided saliva samples for the determination of IL-1β, IL-8, immunoglobulin A (IgA), and CRP. No significant correlations were found between momentary immune fitness and these biomarkers. Further analysis of this dataset by Balikji *et al.*[64] confirmed a significant correlation between ISQ scores and momentary immune fitness (r = 0.452, $p<0.001$). Participants were divided into two groups: an impaired wound healing group (n = 30; self-reported slow healing wounds and/or wound infection in the past year) or a healthy control group (n = 78; self-reported normal wound healing). The impaired wound healing group had significantly lower ISQ scores compared to the control group (5.2 and 6.4, respectively, p=0.009). However, no significant difference was observed between the groups for momentary immune fitness (7.3 and 7.7, respectively, p=0.087). Among the healthy controls, no significant correlations were found between ISQ scores and immune biomarkers. In contrast, for the impaired wound healing group, ISQ scores showed significant positive correlations with IL-1β (r = 0.439, p=0.015) and IL-8 (r = 0.393, p=0.032) (Figure 6). In addition, a significant positive correlation was found between momentary immune fitness and saliva IgA concentrations (r = 0.383, p=0.037).

Authors of two additional studies that assessed biomarkers[65,66] were contacted with a request to share their datasets for further analysis of the relationship between ISQ scores and immune biomarkers. However, no responses were received.

3.5. Randomized controlled trials and other intervention studies

To date, seven experimental studies have included ISQ assessments, with four of these studies conducted in India. Thakar *et al.*[67] conducted a multicenter, prospective open-label study among 20,574 Indian adults (aged 18 – 68 years) who had medium-to-high exposure risk to SARS-CoV-2 infection, such as healthcare workers. Participants were asked whether they would like to receive prophylactic Ayurvedic medication to reduce the risk of SARS-CoV-2 infection. Those who opted for it formed the intervention group (n = 15992) and were administered Guduchi Ghanavati (1 g daily for 28 days) in addition to standard preventive guidelines (SPG), including frequent hand washing, physical distancing, and using face masks. Participants who declined the Ayurvedic medication formed the control group (n = 4953) and received the SPG only. The ISQ and World Health Organization Quality of Life-BREF (WHOQOL-BREF) instrument[68] were administered at baseline and after 28 days. The baseline ISQ scores were 9.3 for the intervention group and 9.14 for the control group, suggesting raw scores were used without converting to the standard 0 (very poor) to 10 (excellent) scale. No significant difference in COVID-19 infection rates was found between the groups. Both groups showed improvements in ISQ scores and quality of life, with a statistically significant but small ISQ increase in the intervention group (+0.30 units) compared to the control

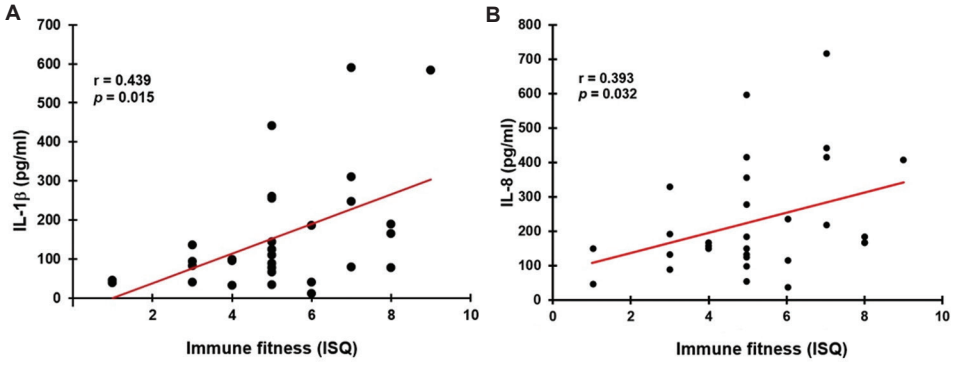

Figure 6. (A and B) Relationship between ISQ scores and saliva immune biomarkers. Data from Balikji *et al.*[64]
Abbreviation: ISQ: Immune status questionnaire.

group (+0.11 units). At baseline, weak but significant correlations (r < 0.25) were found between ISQ scores and all domains of the WHOQOL-BREF. Notably, the ISQ score correlated significantly (r = 0.83) with a single-item general health rating on a 0 – 10 scale.

Nesari *et al.*[65] conducted a prospective, open-label study in India to evaluate the impact of Ayuraksha on immune fitness and SARS-CoV-2 infection rates. A total of 80,000 Delhi police personnel were invited to participate. They received an Ayuraksha kit containing Samsamani Vati, Anu Taila, and Ayush Kwatha. Among them, 45,782 completed a baseline ISQ assessment, with follow-up assessments after 60 days (n = 39,880) and 90 days (n = 32,062) of daily treatment. Surveys were completed in English through Google Forms. The ISQ assessed immune fitness over the past 12 months. Momentary immune fitness was assessed with a single-item assessment on a 0 – 10 scale (Figure 5), while reduced immune fitness was assessed with a yes/no question (Figure 7).

Mean ISQ scores were 9.53 at baseline, 9.61 after 60 days, and 9.54 after 90 days, suggesting self-reported immune fitness was very high (>9). The authors were contacted to clarify whether these scores reflected recoded ISQ values or raw sums and to request further analysis of the dataset; however, no response was received. The lack of significant improvement in ISQ scores over time may be due to a ceiling effect, given the initially high scores. A methodological concern is that ISQ assessments at each follow-up still covered the 12-month period prior, thus overlapping substantially with the pre-intervention phase. This undermines the ability to detect change attributable solely to the intervention. The momentary immune fitness was also very high at each time point: 9.17 at baseline, 9.26 after 60 days, and 9.34 after 90 days. These scores also showed no significant improvement over time. However, the percentage of participants reporting reduced immune fitness progressively decreased from 5.25% at baseline to 3.76% after 60 days and 2.74% after 90 days.

In the second part of the study, a subsample of 101 participants who agreed to take Ayuraksha for 60 days was compared to a control group of 66 participants who declined the intervention. Blood samples were collected

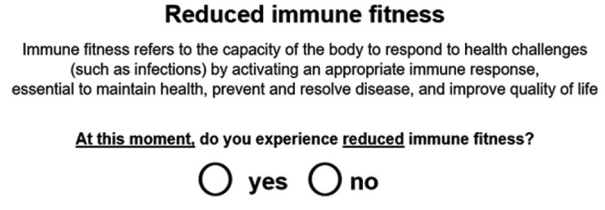

Figure 7. A sample of yes/no binary assessment of reduced immune fitness. Adapted from Verster *et al.*[3]

at baseline and after 60 days for the analysis of immune biomarkers, including CRP, IL-2, -4, -6, -10, and -12, as well as IgG, IgM, IgA, CD3, CD4, and CD8. Immune fitness was assessed using both the single-item scale and the ISQ. The mean age of participants was 39 years, with the majority being male (97.5% of the intervention group and 90.9% of the control group). The percentage of participants who tested positive for SARS-CoV-2 during the study period was significantly lower (p=0.003) in the intervention group (17.5%) compared to the control group (39.4%). ISQ scores in the intervention group improved slightly from baseline (9.30 ± 1.28) to day 60 (9.51 ± 1.08), but the difference was not statistically different. The control group also did not demonstrate statistically significant changes in the ISQ scores. Notably, the ISQ assessed immune fitness for the prior 12 months, thereby including 10 months of pre-intervention data in the day 60 assessment. Similarly, the increase in momentary immune fitness in the intervention group – from 8.84 ± 1.1 to 9.04 ± 1.2 – was not statistically significant nor was any change observed in the control group. These subjective assessments were mirrored by the biomarker data, which showed no significant change in the intervention group. Interestingly, in the control group, IL-6 levels were significantly elevated at day 60. However, the study did not report correlations between perceived immune fitness and immune biomarkers.

In Bengaluru, India, Wadud *et al.*[69] examined the prophylactic effect of a Unani poly-herbal decoction and Khamira Marvareed (herbs-mineral preparation) in 4,500 individuals at risk for SARS-CoV-2 infection. In this randomized, controlled, prospective field study, participants in the intervention group received an oral combination Unnab (*Ziziphus jujube* Mill.), Sapistan (*Cordia myxa* L.), Behidana (*Cydonia oblonga* Mill.), and *Khamira Marwareed* for 20 days, while the control group received no treatment. Assessments were made at baseline and on day 20 through in-person visits, with a telephone follow-up after 35 days. The ISQ and WHOQOL-BREF[68] were used to evaluate immune fitness and quality of life, respectively. Of the total sample, 2,240 participants of the intervention group and 2,073 controls (no intervention) completed the study. Compared to the baseline, the ISQ scores and quality of life improved significantly in the intervention group compared to the control group which showed no significant changes. However, infection rates did not differ significantly between the groups.

Khadke *et al.*[66] conducted a single-blind, randomized, placebo-controlled trial in 28 healthy Indian volunteers to examine the antioxidant and anti-inflammatory properties of a prophylactic polyherbal formulation (PPHF; Kofol immunity tablets). The treatment group (n = 18) received PPHF twice daily for 2 months, while the control group (n = 10) received a matched placebo. The formulation

contained Triphala Guggul, Guduchi (*Tinospora cordifolia*), Haridra (*Curcuma longa*), Manjishtha (*Rubia cordifolia*), Chitrak (*Plumbago zeylanica*), and Trikatu (a combination of *Zingiber officinale*, *Piper nigrum*, and *Piper longum*). Assessments included the ISQ, perceived stress, WHOQOL-BREF, and blood biomarkers such as $CD4^+$, $CD8^+$, IFN-γ, TNF-α, IL-10, malondialdehyde (MDA), and glutathione peroxidase (GPx). PPHF significantly increased ISQ scores, reduced stress, and improved quality of life compared to the placebo group. Biochemical analysis showed decreased MDA, increased GPx, decreased TNF-α, and increased IL-10 in the PPHF group. However, the authors did not provide their dataset, precluding further analysis of correlations between ISQ scores and biomarkers.

In the USA, Stefan *et al.*[70] conducted a randomized, double-blind, placebo-controlled trial to examine the effects of exogenous beta-hydroxybutyrate supplementation – a proxy for a ketogenic diet – on safety and health metrics. Participants in the intervention group consumed 12.75 g of beta-hydroxybutyrate salts twice daily for 90 days, while the control group received a maltodextrin placebo. ISQ scores were assessed at baseline and at 30, 60, and 90 days. No significant differences in ISQ scores were observed between the groups or across time points.

In Malaysia, Goh *et al.*[71] conducted a randomized controlled trial to investigate the effects of combined supplementation with lutein, zeaxanthin, and elderberries on ocular health and immune fitness in 110 healthy volunteers. The intervention group (n = 51) received the product daily for 20 days, whereas the control group (n = 50) received a placebo. Ocular health was assessed using the ocular surface disease index, and immune fitness was assessed with the ISQ. Compared to the placebo group, a significant decrease in ocular complaints was reported by the intervention group. The intervention group also exhibited a 15.9% improvement in ISQ scores (from 7.92 to 9.18). Since the placebo group's ISQ scores also improved (from 7.90 to 8.69), between-group differences in immune fitness were not statistically significant. Correcting sex, age, and BMI as a covariate did not change the findings.

In an open-label study, Harinath *et al.*[72] examined the effects of low-dose naltrexone on immune fitness in 665 American adults with a mean age of 54 years (range: 19 – 96). ISQ was assessed at baseline and after 3 months (range: 89 – 425 days). ISQ scores were recoded according to Wilod Versprille *et al.*,[15] but multiplied by a factor 10 – yield a score range from 0 (very poor) to 100 (excellent). A 16.7% improvement (from 59.3 at baseline to 69.2 after 3 months) in ISQ scores was recorded.

4. Discussion

The studies summarized in this review demonstrate that the ISQ is a valid and reliable tool for assessing immune fitness. The studies confirmed significant correlations between ISQ scores and known factors that can influence immune fitness, including demographics (e.g., sex and age), lifestyle factors (e.g., daily diet, physical exercise, and smoking), mood and personality (e.g., stress, anxiety, and neuroticism), and health correlates (e.g., BMI or presence of underlying disease). An overview of key correlations is presented in Figure 8.

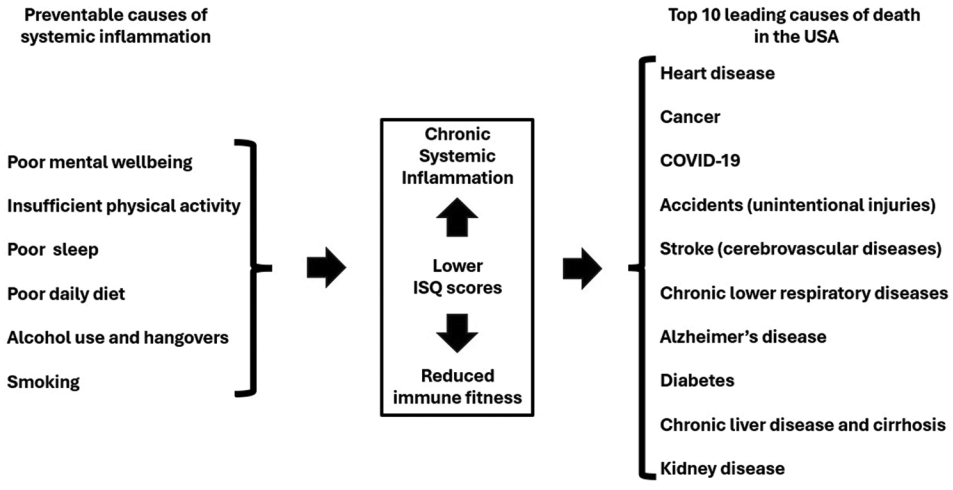

Figure 8. The relationship between ISQ scores, systemic inflammation, and the top ten leading causes of death in the United States. This conceptual model illustrates how preventable lifestyle-related risk factors can lead to chronic systemic inflammation, which is associated with lower ISQ scores and reduced immune fitness. Reduced immune fitness may increase vulnerability to various diseases, including several of the top ten leading causes of death. Abbreviations: COVID-19: 2019 coronavirus disease; ISQ: Immune status questionnaire.

Figure 8 also highlights the top ten causes of death in the United States,[5] many of which are linked to impaired immune fitness. Studies have consistently shown that individuals with these conditions tend to report lower ISQ scores compared to healthy controls. Moreover, individuals who self-report poor immune fitness (through a binary yes/no question) also show significantly lower ISQ scores. Past-year immune fitness scores derived from the ISQ were strongly correlated with momentary assessments. In addition, in patient groups with impaired immune fitness, ISQ scores were significantly associated with biomarkers of systemic inflammation.

4.1. Strengths of the ISQ

In addition to the significant correlations with lifestyle and health outcomes that demonstrate the validity of the ISQ, the questionnaire has several advantages over other measures such as biomarker assessment. First of all, the ISQ is a very short questionnaire. It contains only seven items that are easy to understand and can be completed in under a minute. Unlike biomarker assessments, which are time-consuming and often require costly laboratory facilities, ISQ involves minimal costs and a short duration of testing.[3,4] The ISQ's simple structure – listing common immune-related complaints and their frequency – makes it easily translatable without compromising its psychometric properties. This is supported by successful validation studies in Indonesian populations.[16,17] These advantages make the ISQ a practical tool not only for research but also for routine health screening in clinical and community settings.

4.2. The concept of immune fitness

Immune fitness is a complex concept that is not fully captured by the current version of the ISQ.[4] As demonstrated in this review, various demographics, mood, personality, and health metrics influence overall immune fitness. In addition, there are several characteristics of immune-related complaints next to their frequency of occurrence that has an impact on overall immune fitness. These characteristics are summarized in Figure 9.

As is evident from Figure 9, the ISQ covers only the type and frequency of occurrence of a selected number of immune-related complaints. First of all, there are many more immune-related complaints than the seven items covered by the ISQ. When developing the ISQ, a list of 23 immune-related complaints was reduced to the seven most common complaints that predicted 85% of the total score.[15] The actual frequency of occurrence of the items was not scored due to several reasons. First of all, for several items (e.g., common cold or diarrhea), this could introduce recall bias, which would likely register a lower score when using the five-point Likert scale. Second, some other items (e.g., joint and muscle pain or skin problems) may have fluctuating symptoms, making a numerical scoring less useful. However, the ISQ does not account for several important aspects of immune-related complaints, such as the duration and severity of the symptoms and their impact on daily activities and quality of life.

Theoretically, it is assumed that a single-item overall assessment of immune fitness will automatically take

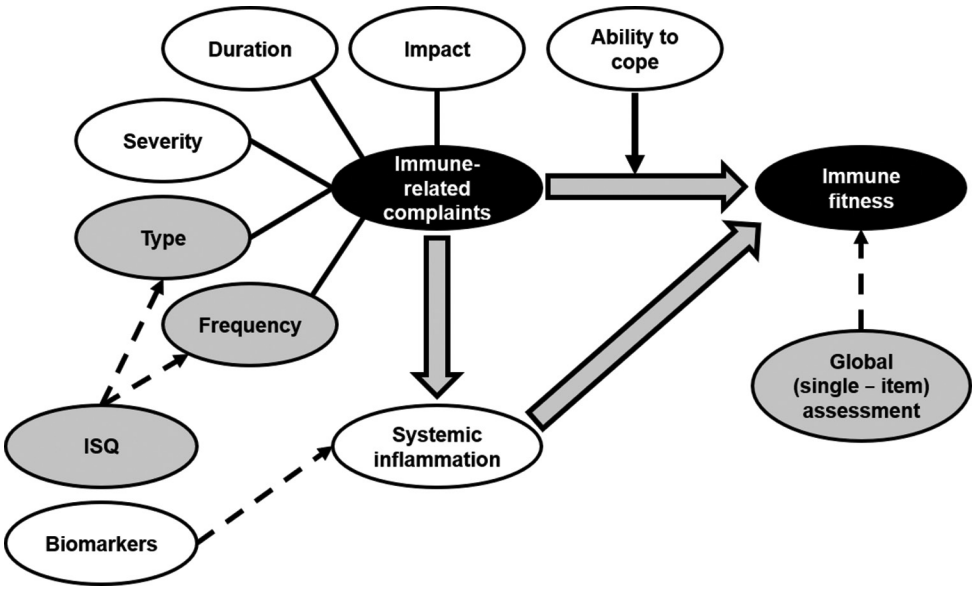

Figure 9. Factors contributing to the perception of immune-related complaints and immune fitness
Abbreviation: ISQ: Immune status questionnaire.

into consideration all factors that are summarized in Figure 9.[4] The US Food and Drug Administration supports the use of single-item assessments over multiple-item questionnaires,[73] as the composite scores from the latter can never provide a complete overarching global assessment of the concept.[4,73] However, although a single immune fitness score theoretically includes all the relevant factors, such an assessment provides no information on the nature, severity, or specific cause of immune-related complaints. Therefore, research is currently in progress to determine if the current ISQ can be improved by incorporating some of these relevant factors. It is also important to recognize the limitations of biomarker assessments. While biomarkers offer objective evidence of inflammation or immune activation, they reflect only a narrow slice of the immune system's functioning. Typically, studies measure a limited panel of biomarkers, which may or may not be indicative of overall immune status. Moreover, many biomarkers provide no information about how immune issues are experienced by individuals – such as the duration or impact of symptoms. Biomarkers can also change without being noticed by patients, similar to asymptomatic hypertension. Therefore, biomarkers should be considered as supportive evidence for the assessment of overall immune fitness, but not vice versa.

4.3. Scoring of the ISQ

Another aspect of the current ISQ that might need revision in the future is its scoring system (Figure 2). The current scoring of items ranges from 0 to 4, yielding a raw sum score from 0 to 28, which is then recorded into a score of 0 (very poor) to 10 (excellent). At first glance, this final score is easy to interpret. For example, in the validation article,[15] scores below 6 were interpreted as indicating reduced immune fitness. However, after using the ISQ in various studies and discussing the outcomes with both study participants and academic colleagues, it appears that a cut-off score is not warranted. The latter is also true for single-item assessments of overall immune fitness.[3] Immune fitness is a relative perception that varies by individual. For example, an elite athlete may report a baseline score of 9, corresponding to peak performance. If they later report a 7, this reflects a perceived reduction in immune fitness – even though 7 is still considered a "healthy" score. Thus, a score interpreted in isolation may not accurately reflect a change in health status. To address this, researchers are encouraged to assess reduced immune fitness separately using a simple yes/no question.

Another issue with the current scoring approach is that all seven immune-related complaints are weighted equally. However, some of the complaints may have a greater impact on overall immune fitness and well-being than others. For example, having a headache is likely to have a bigger negative impact on daily activities than coughing. Furthermore, scores of some items correlate with each other, while others do not.[15,16] Therefore, previous studies have proposed differentially weighting the scores of the ISQ items.[16] Future research should determine whether this strategy will result in a relevant improvement of the ISQ.

As illustrated in Figure 9, overall immune fitness is influenced by more than just the type and frequency of seven specific complaints. This broader complexity likely explains why correlations between ISQ scores and single-item overall immune fitness scores are not perfect ($r = 0.4$).[3] Hence, it is important to re-evaluate the current ISQ scoring format, considering possible adaptations to better match global immune fitness assessments.

4.4. Recommended use of the ISQ in experimental studies

In experimental studies, the ISQ is very suitable for assessing baseline or pre-intervention immune fitness. However, it is important to recognize that the ISQ reflects the past year's immune fitness. If the goal is to measure current or momentary immune fitness, other assessment tools such as the single-item immune fitness scale (Figure 5) or the yes/no reduced immune fitness question (Figure 7) are more suitable.

The original ISQ measures the occurrence of immune-related complaints over the past 12 months. Depending on the study design, this time period can be adjusted. For example, it can be reduced to the past three or 6 months. Naturally, the shorter the period assessed, the fewer complaints participants are likely to report – thus yielding higher ISQ scores. Conversely, if the recall period is too short, participants may not have experienced any complaints, which limits the questionnaire's informativeness. We propose that the minimum recommended timeframe for the ISQ should be 4 weeks. However, this needs to be validated in prospective studies, which should also explore whether the scoring system should be adjusted for shorter recall periods (e.g., reporting the number of days a complaint was experienced instead of using a Likert scale). If assessments are needed for very short timeframes (e.g., <1 month), a better alternative may be the single-item immune fitness scale. This tool allows researchers to modify the reference period (e.g., "At this moment" or "During the past 2 weeks") to capture more immediate perceptions of immune fitness.

In the experimental studies reviewed here, the ISQ was administered without adjusting its 12-month recall period. This approach introduces bias in the study outcome. For example, if the ISQ is completed at baseline (visit 1), and again at 30 days (visit 2), 60 days (visit 3), and 90 days (visit 4), subsequent assessments still reflect immune

complaints experienced before the intervention began (i.e., 9 – 11 months prior). If the goal is to monitor monthly changes, a tool designed for shorter recall – such as a past-month single-item immune fitness assessment – would be more appropriate.

4.5. Use of the ISQ in clinical practice

Although not documented in scientific literature, the ISQ is also used in clinical practice. An online search revealed various examples of physicians using the ISQ for screening or educational purposes.[74,75] For example, the Arizona Family Health Centre offers the ISQ on its website,[74] alongside blogs and a video explaining immune health and strategies for improvement. In cases where patients score poorly – indicating reduced immune fitness – further medical evaluation may be warranted. However, it is important to note that such websites clearly state that the ISQ is used for informational and educational purposes only and is not intended to provide medical advice or replace consultation with a healthcare professional.

Another example comes from the Oak Wellness Hub from Ghana, Africa, which used the ISQ to increase public awareness of immune health during the COVID-19 pandemic.[75] Their website combined the ISQ with lifestyle-related questions, enabling patients to evaluate their immune status. The website invited people with the tagline: "discover your immune status in <5 min." One patient who completed the questionnaire reported: "*This questionnaire helped allay my fears when the COVID-19 pandemic hit. When I got a suboptimal score. I was alarmed. I thought I was doing so well in keeping healthy. This great tool and its rich resources educated me on what to do and strengthened my resolve to boost my immunity.*"[75] Of importance, the website emphasized that their questionnaire was intended as a screening guide and not a replacement for professional medical assessment.

Online screening tools like these can help patients evaluate their immune fitness and make informed decisions about seeking medical attention or adjusting their lifestyle. Importantly, the ISQ is freely available for public use and distribution, making it a valuable tool for individuals and countries with limited financial resources.

5. Conclusion

In the 5 years since Wilod Versprille *et al.*[15] developed the ISQ, a total of 38 studies (51 publications) have implemented the questionnaire in their research. These studies have shown that the ISQ is a reliable, valid, and cost-effective tool. It is also an easy-to-understand questionnaire consisting of seven items that can be completed within a short time. The ISQ has been implemented in different types of studies and clinical settings and has been translated and validated in different languages.

The studies described in this review demonstrated that the ISQ scores are related to demographics (e.g., sex and age), mood, personality, disease states, health correlates, and lifestyle factors. In addition, ISQ scores exhibited a correlation with immune biomarker concentrations. The ISQ has been used in multiple cross-sectional surveys and a limited number of experimental studies – either to assess baseline immune fitness or monitor intervention effects such longitudinal studies, the original 12-month recall period may be adjusted to a shorter time period. Based on the current review, a minimum recall period of 4 weeks may be appropriate, though further research is needed to confirm this. For shorter periods, the single-item global immune fitness scale is recommended instead.

Beyond research, the ISQ is increasingly being used in clinical practice for routine screening. It offers patients and clinicians a quick, affordable, and personalized snapshot of immune health, which can help guide decisions on whether further evaluation (e.g., lifestyle assessment, physical examination, or biomarker testing) is warranted.

Future research should explore potential improvements of the ISQ, such as incorporating additional aspects of immune-related complaints such as duration, severity, and impact and refining the scoring system to better capture overall immune fitness. Notwithstanding these opportunities for improvement, the ISQ has already established itself as a practical and informative tool for assessing immune fitness in both research and clinical settings.

Acknowledgments

None.

Funding

None.

Conflict of interest

Over the past 3 years, Joris Verster has received research support from Danone and Inbiose and acted as a consultant/advisor for Eisai, KNMP, Med Solutions, Mozand, Red Bull, Sen-Jam Pharmaceutical, and Toast. The author also owns stock in Sen-Jam Pharmaceutical. Joris Verster and Emina Išerić received travel support from Sen-Jam Pharmaceutical. Johan Garssen is a part-time employee of Nutricia Danone and received research grants from Nutricia Research Foundation, Top Institute Pharma, Top Institute Food and Nutrition, GSK, STW, NWO, Friesland Campina, CCC, Raak-Pro, and EU. However, these have not influenced the content of the manuscript.

Author contributions

Conceptualization: All authors
Visualization: Joris Verster
Writing–original draft: Emina Išerić, Joris Verster
Writing–review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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