

Ageing impairs parasite-specific antibody responses in cystic echinococcosis

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Summary

Humoral immunity wanes during healthy ageing, increasing susceptibility to infections in the elderly. In this sense, information about parasite infections and human immunosenescence is scarce. Cystic echinococcosis (CE) is an infectious disease caused by the larval stage of the cestode parasite *Echinococcus granulosus*, whose prevalence in humans shows an increase with host age. Susceptibility to human CE has been associated with humoral immunity to some extent, and, therefore, we have here analysed the influence of host age on the serological profile of young, middle-aged and aged patients. Our results highlighted the detrimental influence of ageing on the intensity and quality of the antiparasite antibody response. Remarkable differences in serological profiles between young and aged individuals were observed. In this sense, through Principal Components Analysis, we identified aged patients as those exhibiting overall less intense antibody responses, mainly in isotypes/subclasses supposed to exert efficient antiparasite activities (eg IgE and IgG1). Thus, these humoral defects could at least partially explain the reported increase in CE prevalence among older individuals, as a weaker immune response in the elderly might facilitate the establishment and maintenance of the parasite infection. Finally, a possible association between age-dependent susceptibility to CE and host immunosenescence is discussed.

KEYWORDS

ageing, antibodies, cystic echinococcosis, *Echinococcus granulosus*, susceptibility

1 | INTRODUCTION

Cystic echinococcosis (CE) is a parasitic zoonosis with cosmopolitan distribution caused by the larval stage of the cestode *Echinococcus granulosus*. Dogs are the most common definitive host for the parasite and adult worms reside within their intestines, producing eggs containing oncospheres that are spread into the environment through faeces. Cystic echinococcosis occurs in intermediate hosts (mainly domestic and wild ungulates, with humans acting as accidental hosts) and derives from the ingestion of oncosphere-containing eggs, which later develop into metacestodes (hydatid cysts) mainly in the liver and lungs of the infected host.^{1,2}

Prevalence of human CE depends on several factors, being host age one of them. Worldwide data obtained from systematic literature

reviews, hospital discharge records and/or field surveys have shown that prevalence increases with host age.³⁻⁷ For example, in a prospective systematic review on CE risk factors in the Qinghai-Tibet Plateau (China), a positive linear relationship was reported between prevalence and increasing age.⁸ Additionally, in an Italian study based on hospital discharge records, among 10 682 CE patients with age at diagnosis ranging from 1 to 100 years old, only 7% were younger than 30 years old, whereas 57% were aged over 60 years old.⁹ Similar results were reported recently in western Spain based on data obtained from the public healthcare system during the period 2000-2012.¹⁰

Cystic echinococcosis diagnosis mainly relies on ultrasound results, having serological tests a complementary role with performances strongly influenced by the antigen and the technique used, as well as by many clinical variables.^{11,12} In this sense, parasite-specific

antibodies in seropositive patients differ when grouped according to infection stage,^{13,14} primary vs relapsed disease¹⁵ or chemotherapy responsiveness.¹⁶ Therefore, this study aimed at analysing whether host ageing influences the serological profile in patients with surgically confirmed CE.

2 | MATERIALS AND METHODS

To establish the relationship between *E. granulosus*-specific antibodies and the age of the patient, we re-analyse our own previously reported data on parasite-specific values (antiprotoscolex somatic antigens, PSA) for IgM, IgE, IgA subclasses and IgG subclasses in 22 patients with surgically confirmed CE.¹⁷ Samples belonged to the serum bank stored at -80°C reported in,¹⁷ according to Ethics Statements (ethical committee approval and patients consents) described therein. In the present work, those patients were clustered into three groups: young patients (<35 years old; four females and three males; median age: 23, range: 12-34), middle-aged patients (35-55 years old; four females and three males; median age: 43, range: 35-49) and aged patients (>55 years old; three females and five males; median age: 63, range: 58-78). Groups were selected in a way that similar age ranks (roughly 22 years per group) were covered over the complete age range of the samples (from 12 to 78 years old). Additionally, no bias regarding female-to-male ratios was observed. Unfortunately, no additional clinical information other than gender and age was available.

Statistical differences between groups were analysed by nonparametric Kruskal-Wallis test and correlation analyses between antibody

levels and patients' age were assessed by Spearman's rank correlation test. In all cases, differences and correlations were regarded as significant with P -value $< .05$.

Finally, to identify serological patterns able to differentiate young and aged patients with CE, we performed Principal Component Analyses (PCA).¹⁸ To that end, profile data from PSA-specific isotypes/subclasses were converted into a matrix of 15 objects (seven young and eight aged patients with CE) and eight variables (PSA-specific titres of IgM, IgE, IgG subclasses and IgA subclasses). Data were scaled when necessary to work with values in the same order of magnitude, and analyses were based on correlation matrices. Factor weights (loadings) for the two principal components (PC) showing the largest eigenvalues are shown in Figure 1. Loadings $> |0.750|$ were considered the most relevant for the analyses.

3 | RESULTS

In the first place, to determine the influence of host ageing on *E. granulosus*-specific antibodies, we performed a set of correlation analyses between antibody levels and the age of patients with CE. Our results showed a negative and significant correlation for most isotypes/subclasses: IgM (r^2_S : -0.6350 , $P = .0015$), IgE (r^2_S : -0.5125 , $P = .0147$), IgG1 (r^2_S : -0.4985 , $P = .0182$), IgG2 (r^2_S : -0.5157 , $P = .0140$), IgG4 (r^2_S : -0.4434 , $P = .0388$), IgA1 (r^2_S : -0.6706 , $P = .0006$) and IgA2 (r^2_S : -0.6073 , $P = .0027$). Interestingly, only PSA-specific IgG3 levels showed a nonsignificant correlation with the age of the host (r^2_S : -0.3130 , $P = .1561$). Overall, these results indicate that the older the patient, the lower the levels of most parasite-specific antibodies;

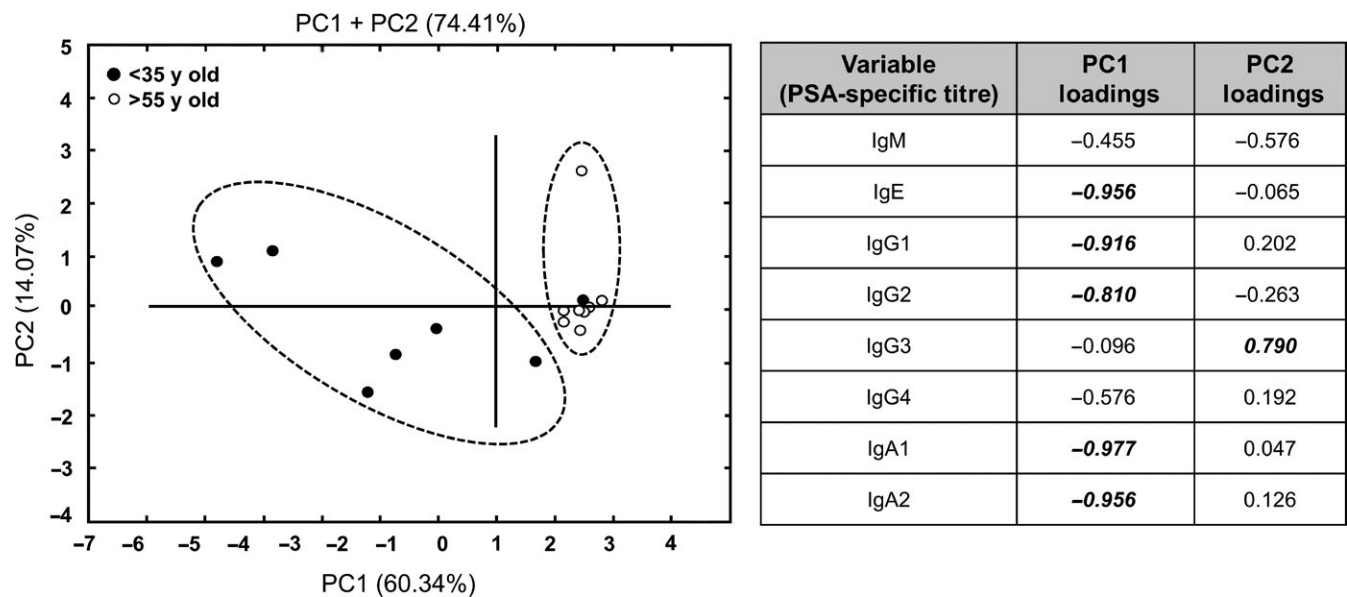


FIGURE 1 Principal Components Analysis of young and aged patients with Cystic echinococcosis (CE). Studies of Principal Component Analyses (PCA) for PSA-specific serological profiles in young and aged patients with CE are shown. Factor loadings for the two PCs with the largest eigenvalues are shown, and the most relevant for the study ($> |0.750|$) are highlighted with bold italic letters. Young and aged patients are depicted as black and white circles, respectively. PCon vs PC2 biplot showed that aged patients clustered away from most young individuals. In this sense, PCon explains differences between age-grouped patients and showed that aged patients with CE are those exhibiting the lowest levels of PSA-specific IgE, IgG1, IgG2, IgA1 and IgA2 antibodies

suggesting a probable detrimental influence of host ageing on the antiparasite antibody response mounted by patients with CE.

Then, we further performed a detailed analysis by grouping patients with CE into three age-dependent categories: young (<35 years old), middle-aged (35–55 years old) and aged (>55 years old). Results in Table 1 showed that median PSA-specific values for IgM and IgE were significantly lower in aged patients than in young individuals (three and 40 times lower, respectively). On the other hand, regarding PSA-specific levels of IgG subclasses, results in Table 1 showed again the most relevant differences between young and aged patients. In this case, IgG1, IgG2 and IgG4 levels in aged patients were approximately 380, seven and 160 times lower than in young individuals. Interestingly, no significant differences among groups were observed for PSA-specific IgG3 levels. Regarding parasite-specific IgA subclasses, results in Table 1 showed a very similar behaviour to PSA-specific IgG2; IgA1 and IgA2 exhibited both roughly sevenfold lower median levels in aged patients with respect to young individuals. Thus, our results highlighted the existence of remarkable differences in parasite-specific antibody profiles according to the age of the patients, being such differences maximized between young and aged patients.

Finally, we performed a PCA for PSA-specific serological profiles in young and aged patients with CE. Principal Component Analyses is an algorithm of descriptive statistics commonly used to reduce the number of attributes used to represent a data set in order to identify patterns that stand out their similarities and differences. The procedure uses orthogonal transformations to convert the observations, which are presented as functions of possibly correlated variables, into a set of values of linearly uncorrelated variables called principal components (PC). We constructed a matrix for PCA including PSA-specific titres of IgM, IgE, IgG subclasses and IgA subclasses, yielding eight PCs. PC1 and PC2 accounted together for 74.41% of the total variability among individuals, and the biplot of PC1 vs. PC2 showed that aged patients clustered away from most young individuals (Figure 1). Accounting for 60.34% of the total variability, PC1 explains differences between age-grouped patients along the x-axis in the scoring plot. Analysis of the loading factors of PC1 showed that this component includes most of the isotypes/subclasses studied with remarkable negative loadings, while no variables in PC1 exhibited positive loadings (Figure 1). Accordingly, in moving from left to right in the biplot, aged patients

with CE are those expressing the lowest levels of PSA-specific IgE, IgG1, IgG2, IgA1 and IgA2 antibodies. On the other hand, PC2 accounted only for 14.07% of the total variability, mainly discriminating between individuals within each group, having in this case PSA-specific IgG3 levels the greatest loading factor (Figure 1). In summary, our PCA study suggests that aged patients with CE develop a significant less intense antibody response, mainly in isotypes/subclasses supposed to exert better antiparasite activities (e.g IgE and IgG1).

4 | DISCUSSION

Healthy ageing is accompanied by well-documented physiological changes. Among them, alterations in immune function with increasing age are profound and can be observed across the whole immune system.^{19,20} The functional changes reported in immune ageing—known as immunosenescence—have relevant effects on the global physiological status of the individual, being increased susceptibility to infections one of the most important.^{20,21}

Regarding parasite infections and human immunosenescence, there is little information.²² In this sense, in a human population in Cameroon, a Th2-type phenotype was associated with greater resistance to *Ascaris lumbricoides* and *Trichuris trichiura*, a response that increases in magnitude until 18 years of age before declining significantly, suggesting that older individuals have less effective defences.^{23,24} Regarding infections by cestodes—the less studied group of helminths—there is a report on cysticercosis due to *Taenia solium* infection pointing at ageing as an influencing factor on human susceptibility to the infection.²⁵ In that work, performed in an endemic rural area of Ecuador, it was shown that although the proportion of seropositive individuals significantly increased with age until 40 years old (becoming then stable in older individuals), the proportion of people presenting circulating parasite antigens—reflecting an active infection—was significantly higher in people older than 60 years old. Such observation was ascribed by the authors to immunosenescence, as a weaker immune system in the elderly would facilitate the establishment and maintenance of viable cysticerci compared with fully immunocompetent young individuals.²⁵

In this regard, our results highlighted the detrimental influence of host ageing on the anti-*E. granulosus* antibody response, with

TABLE 1 Antiparasite antibody levels according to age group. Levels of anti-PSA IgM, IgE, IgG subclasses and IgA subclasses were determined through ELISA. Median values and ranges are shown for the three groups under study

PSA-specific titer Median (AU/mL) (range)	<35 years old	35–55 years old	>55 years old
IgM	31 659* (11 474–80 913)	25 468* (22 131–35 937)	12 300 (2973–29 351)
IgE	5443* (24–8038)	1300 (6–7187)	137 (6–1397)
IgG1	12 441* (22–53 338)	1086 (22–8853)	33 (22–1294)
IgG2	26 531* (4348–51 429)	5978 (1701–26 054)	3810 (489–16 344)
IgG3	14 253 (23–67 966)	7571 (805–66 310)	1322 (552–225 000)
IgG4	4492* (22–183 817)	5772 (11–18 248)	29 (11–8231)
IgA1	39 599* (6584–104 154)	10 555 (3063–34 978)	5552 (1688–12 715)
IgA2	37 165* (5332–111 434)	17 212 (1769–32 882)	5145 (1028–12 111)

*Statistical significance ($P < .05$) respect to aged patients.

remarkable differences in antibody profiles according to the age of the patient. Overall, our results suggested that aged patients with CE develop significantly less intense antibody responses, mainly in isotypes/subclasses supposed to exert better antiparasite activities (e.g. IgE and IgG1). This defect in antibody responses could at least partially explain the reported increase in CE prevalence among older individuals, basically due to the induction of less efficient humoral effectors.

Finally, it is important to note that our work may exhibit some limitations due to the small sample size—which could affect population representativeness—and the lack of other clinical data than host age and gender. In this sense, unavailable additional information—such as parasite genotype, cyst location, parasite size and viability, among others—might also contribute to the age-dependent prevalence of human CE, and therefore, they cannot be discarded as age-related influencing factors. However, our results and the above-mentioned reports on age-dependent susceptibility to helminth infections suggest that host immunosenescence might play a role in determining infection susceptibility to human CE.

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CONFLICT OF INTERESTS

All authors declare that there is no potential conflict of interests relevant to this article to declare.

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