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ORIGINAL ARTICLE

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# The prevalence of human thymic lymphoid follicles is lower in suicides

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**Abstract** In an autopsy study we determined the prevalence of thymic lymphoid follicles in 311 accident victims in whom the time interval between accident and death was known. We found that the prevalence decreased abruptly in those surviving 48 h or more ($P$=0.000008). We then compared the prevalence in 271 accident and 168 suicide victims, all of whom had died less than 48 h after the incident and found that the prevalence was significantly lower in the suicide group ($P$=0.03). We conclude that this difference may be related to the effect on the thymus of high levels of psychological stress likely to have been experienced by the suicides in the days prior to the act. The use of the term hyperplasia to indicate the presence of lymphoid follicles in the thymus and the methodology appropriate for determining the prevalence of thymic lymphoid follicles are discussed.

**Key words** Human thymus · Lymphoid follicles · Suicide · Accidental death · Psychological stress · Thymic hyperplasia

## Introduction

It has long been accepted that stress can affect both the structure [7, 23] and the function [18] of the immune system. These effects are achieved via complex interactions between the neuroendocrine and immune systems [4, 5, 17, 20, 21, 26, 27]. Laboratory animals have shown morphological changes in immune tissues and altered immune function in response to both organic and psychological stressors [6]. In human subjects, alterations in peripheral immune function have been demonstrated in response to specific psychological stressors such as bereavement [3], exam stress [11], stress associated with caring for relatives with Alzheimer's disease [12], endogenous depression [22] and cancer stress [1]. Information about the effects of stress on the morphology of the human thymus has been limited to that gained from autopsy studies. These have shown a decreased volume of the thymic cortex [9] associated with an increased level of apoptosis (termed pyknosis in early studies) of cortical lymphocytes [2, 8, 16] and decreased prevalence of thymic (B) lymphoid follicles [14, 24] consequent on the stress of organic illness or injury.

The aim of this study was to determine the effects of psychological stress per se on the prevalence of lymphoid follicles in the human thymus. Using data from apparently otherwise healthy individuals dying at known intervals after accidents, we demonstrated a temporal relationship between the onset of stress and the prevalence of thymic lymphoid follicles. We then investigated individuals who had died in the interval after an accident or an act of suicide, before a consequent stress-related decrease in the prevalence of these follicles would have been anticipated. We believe that the significantly lower prevalence of follicles in the suicide group than in the accident group is likely to be due to major psychological stress present for some days before the act of suicide.

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## Materials and methods

Thymuses were collected at forensic autopsies on 311 accident and 168 suicide victims. These did not include 53 cases in which a distinction between suicide and accident was not possible. No evidence of autoimmune disease was detected in any of the subjects included in the study. All 311 accident cases were included in the part of the study assessing the effect of duration of the terminal condition on the prevalence of lymphoid follicles, but only the 271 accident victims who had survived for less than 48 h were included in the part of the study comparing lymphoid follicle prevalence in accidents and suicides.

The thymuses were fixed in 10% buffered formalin or formol acetic alcohol, and four generous blocks were cut from each. One section from each block was stained with H&E, and in one or more blocks per case one adjacent section was incubated with the monoclonal antibody L26 (DAKO) .The sections were viewed by workers who had no knowledge of the clinical details of the cases.

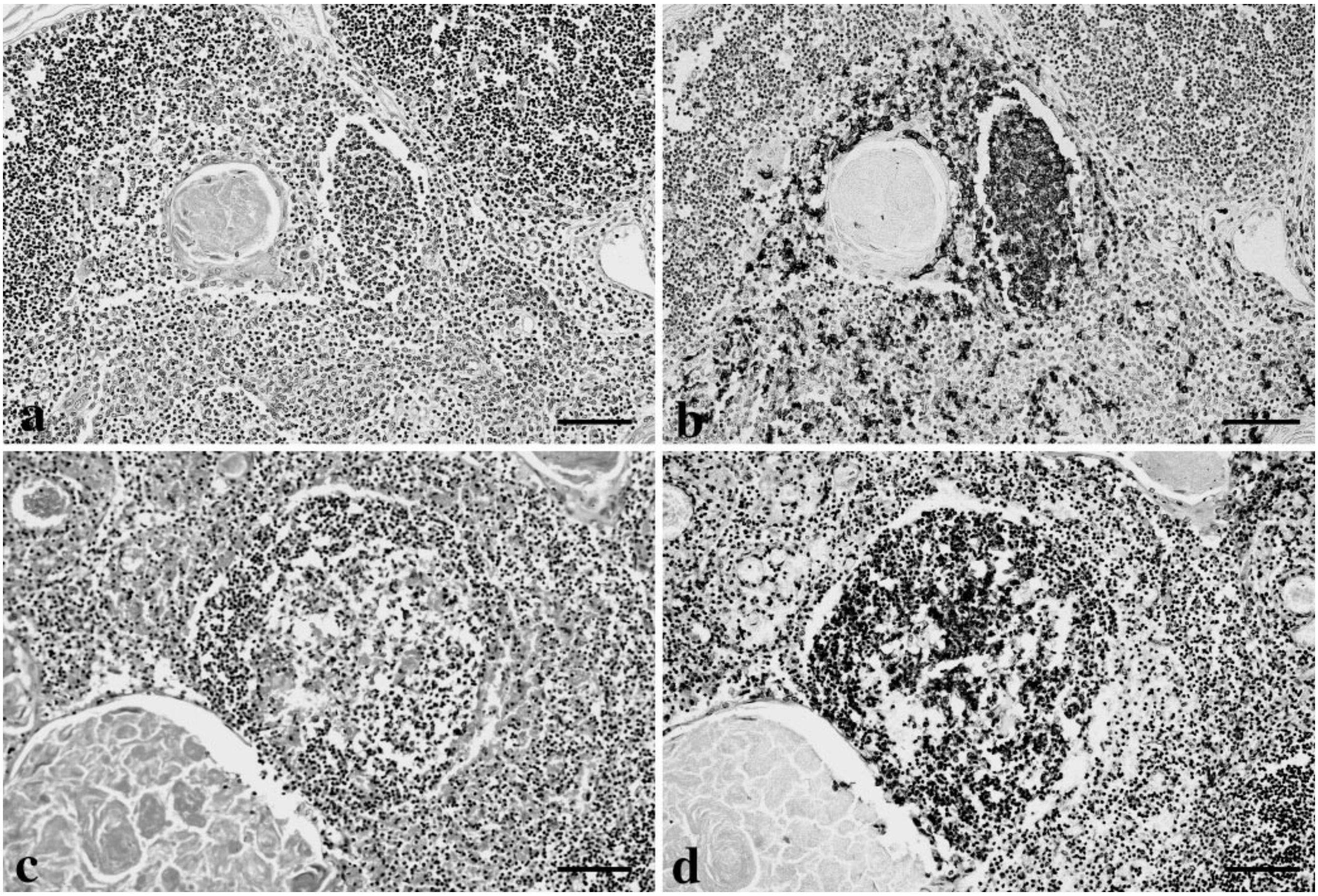


**Fig. 1a–d** H&E stains and L26 labelling of thymic follicles in **a**, **b** a 21-year-old man and **c**, **d** a 19-year-old woman. The L26 antibody labels the follicles and scattered B cells in the adjacent parenchyma. ×192. *Scale bars* 80 μm in each case

Structures that were counted as lymphoid follicles were rounded structures similar in appearance to those seen in lymph nodes. Some of them had pale centres in the plane of section, whereas others showed a uniform appearance, being composed mainly of lymphoid cells with nuclei larger than those predominating in the cortex and more tightly packed than those in the medulla. They were labelled by L26 antibody (Fig. 1) The number of follicles identified in four sections (one from each block) from each case was recorded as the follicle count.

The weight of the thymus at autopsy was recorded in 266 of the 439 cases included in the accident–suicide comparison study.

A previous study showed considerable variation in the prevalence of thymic lymphoid follicles according to the age of the individual [15]. From no lymphoid follicles up to the age of 12 months, the prevalence rose rapidly to reach a peak of 93% in the first half of the second decade then declined gradually to be only 9% in the age group greater than 60 years. We divided our cases into age groups, which reflect these natural variations.

We used the Mantel-Haenszel method to compare the prevalence of follicles in the different study groups after stratifying by age group. We also used this method to compare prevalence of follicles in males and females. Analysis of variance was used to compare mean thymus weights across groups, and the nonparametric Kruskal-Wallis test was used to compare follicle counts because the distribution of counts was highly skewed.

The study on which this paper is based was approved by the Medical Research Ethics Committee of the University of Queensland.

## Results

The abrupt decrease in the prevalence of thymic lymphoid follicles in those surviving 48 h or more after an accident is shown in Fig. 2. The same data divided by age groups is shown in Table 1 (Chi-square 19.89; $P=0.000008$). The prevalence by age group in suicides and in accidents is shown in Fig. 3. The prevalence is significantly higher in accidental deaths than in suicides (Chi-square 4.38; $P=0.03$). No statistically significant difference was found in the values obtained for prevalence of follicles for males and females ($P>0.05$). There is no statistically significant difference in the values obtained for the two categories with regard to follicle counts per thymus in those positive for follicles (Table 2) or thymus weights (Table 3). The individual follicle counts in both groups show considerable variation, ranging from 1 to 116 in suicides and 1 to 235 in accident victims.

## Discussion

This study suggests that psychological stress can be responsible for a decrease in the prevalence of human thymic lymphoid follicles. Previous studies have found a lower prevalence of thymic lymphoid follicles in subjects dying after some time in hospital than is found in

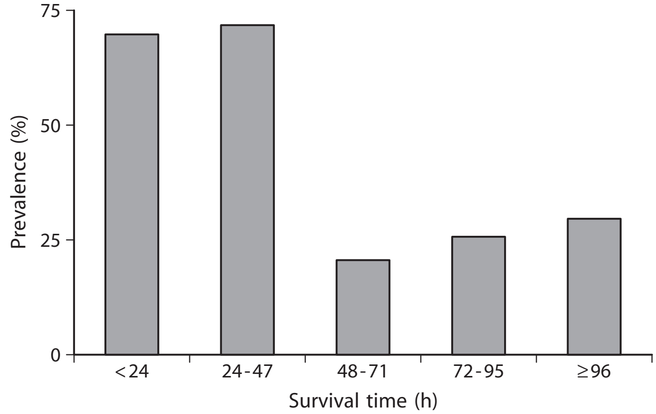


**Fig. 2** Accidental deaths: change in prevalence of thymic lymphoid follicles with increasing survival time. Age ≥10 years; *n*=311

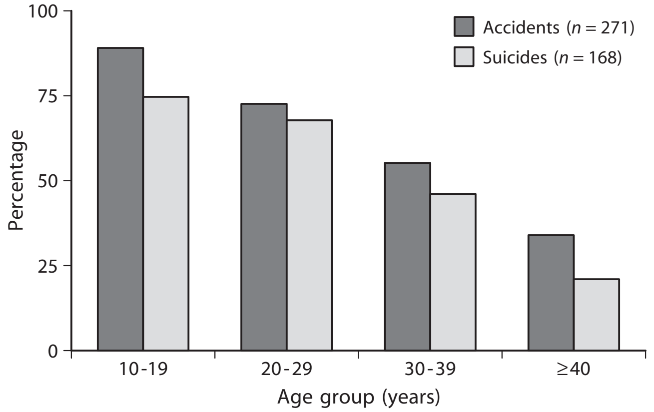


**Fig. 3** Prevalence of thymic lymphoid follicles – accidents vs suicides; survival time <48 h. Mantel-Haenszel stratified analysis of four tables: Chi-square 4.52; *P*-value=0.03

**Table 1** Accidental deaths – prevalence of thymic lymphoid follicles: survival (<48 h versus survival ≥48 h) by age group

| Age group | Prevalence-survival <48 h | Prevalence-survival ≥48 h |
|---|---|---|
| 10–19 years | 89% (*n*=87) | 55% (*n*=9) |
| 20–29 years | 73% (*n*=97) | 40% (*n*=10) |
| 30–39 years | 55% (*n*=43) | 16% (*n*=12) |
| ≥40 years | 34% (*n*=44) | 0% (*n*=9) |

Mantel Haenszel stratified analysis for four tables: Chi-square 19.89; *P*-value=0.000008

**Table 2** Follicle counts: median follicle count by age groups for thymuses positive for follicles. Kruskal-Wallis test for two groups shows no significant difference between accidents and suicides (*P*>0.05) for each age group and for all ages combined (*iqr* interquartile range, *r* range of counts)

| Age group | Median count: accidents | | Median count: suicides | |
|---|---|---|---|---|
| 10–19 years | 15 | *n*=74<br>iqr=5 to 27<br>*r*=1 to 122 | 13 | *n*=15<br>iqr=4 to 26<br>*r*=1 to 102 |
| 20–29 years | 8 | *n*=71<br>iqr=3 to 14<br>r=1 to 43 | 5.5 | *n*=46<br>iqr=3 to 10<br>r=1 to 116 |
| 30–39 years | 5 | *n*=23<br>iqr2 to 10<br>r=1 to 35 | 11 | *n*=19<br>iqr=4 to 21<br>r=2 to 49 |
| ≥40 years | 5 | *n*=15<br>iqr=2 to 8<br>r=1 to 235 | 2 | *n*=7<br>iqr=1 to 6<br>r=1 to 42 |

**Table 3** Thymus weights. ANOVAR test for two groups shows no significant difference between suicides and accidents (*P*>0.05) for either age group or for all ages combined (*n*=number of cases, *SD*=standard deviation)

| Age group | Mean weight, accidents | Mean weight, suicides |
|---|---|---|
| 10–19years | 31 g (*n*=62; SD=14) | 34 g (*n*=15; SD=15) |
| 20–29 years | 25 g (*n*=53; SD=10) | 23 g (*n*=44; SD=9) |
| 30–39 years | 20 g (*n*=27; SD=8) | 20 g (*n*=19; SD=8) |
| ≥40 years | 26 g (*n*=21; SD=14) | 21 g (*n*=25; SD=11) |

those dying suddenly [14, 24]. These studies suggested that this decreased prevalence is a stress effect and that the stress has to be present for some days. We determined the prevalence of follicles in groups of accident victims dying in successive 24-h periods after an accident and found that the prevalence declined abruptly in those surviving 48 h or more (Fig. 1, Table 1). It follows that the significant difference in prevalence found between the suicide victims and the age-matched accident victims (both groups dying less than 48 h after the fatal incident) is likely to be due to some factor affecting the suicide victims and not the accident victims. We believe that this factor is high levels of psychological stress in the days leading up to the suicide.

The wide range of counts of follicles in both categories (Table 2) together with the high prevalence in both categories (the prevalence for accidental deaths being of the same order as that quoted for thymuses from myasthenia gravis patients) is consistent with the concept that B-lymphoid tissue is a normal constituent of the human thymus [10, 15, 28] and that the quantity of lymphoid follicles in the thymus, as elsewhere, represents the result of a balance between antigenic stimulation and immunosupressive factors. The antigen(s) involved can be ‘non-self’ and a component of a normal immune response or ‘self’ and a component of an autoimmune process. The findings serve to reinforce the previously indicated need for caution in attaching pathological significance to the mere presence of lymphoid follicles in the human thymus [14, 15, 28] and in the use of the term thymic hyperplasia [13] if any follicles are discovered in sections of a thymus.

The failure to demonstrate differences in thymic weights between the two groups (Table 3) is not surprising. A previous study on individuals who had suffered a sudden death [25] recorded the weights of thymuses and in addition measured the proportion of thymic volume occupied by cortex. Both variables showed high standard deviations within age groups. Their findings show that there is little age variation in thymic weights but considerable reduction in the proportion of thymus occupied by cortex with increasing age. For example, thymuses from individuals in the 20- to 24-year age group showed a mean cortical volume of 28.5% (SD=10.2), those in the 30- to 39-year age group a mean of 11.7% (SD=8.1) and those in the 70- to 79-year age group a mean of 2.5% (SD=1.8). As the stress-related decrease in thymic weight can be attributed chiefly to a decrease in cortical volume [9] it seems likely that all but major weight changes could be masked by the wide natural variation in cortical volume and in thymic weights.

Attention has previously been drawn [15, 19] to the wide variation in the reported values for the prevalence of thymic lymphoid follicles in subjects chosen as controls in studies on autoimmune diseases. We believe that protocols for such studies should make allowances for age variations and the effects of stress, and that a standard number of blocks should be cut per thymus.

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