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Structural analysis of the thymic cortico-medullary ratio and lymphocyte distribution in age-related involution

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Abstract

Background: Age-related thymic involution contributes to immunosenescence through progressive structural and cellular alterations. This study quantified changes in the corticomedullary (C:M) ratio, thymocyte distribution, tissue composition, proliferative activity, and Hassall's corpuscle morphology across different age groups. **Methods:** Fifty thymic autopsy specimens (five age groups; n = 10/group) underwent histological and immunohistochemical staining (H&E, Masson's trichrome, pan-cytokeratin, CD3, and Ki-67). Digital morphometry quantified the C:M ratio, thymocyte density, tissue composition, Ki-67 index, and Hassall's corpuscle morphology. **Results:** The C:M ratio declined significantly from 3.1 ± 0.4 in the youngest group to 0.7 ± 0.2 in the oldest ($p < 0.001$). Cortical and medullary thymocyte densities decreased by 75% and 54%, respectively, while Ki-67-positive cortical thymocytes declined from 18.6% to 3.1%. Thymic parenchyma decreased from 88.4% to 19.8%, accompanied by an increase in adipose tissue from 6.8% to 76.9%. Hassall's corpuscles enlarged with age, although their density declined in older individuals. Age correlated negatively with the C:M ratio ($r = -0.93$) and positively with adipose tissue area ($r = 0.94$). **Conclusion:** Progressive thymic involution is characterized by cortical atrophy, reduced thymocyte proliferation, and adipose replacement, supporting the corticomedullary ratio as a reliable histomorphometric marker of thymic aging.

Keywords: *Thymus, Cortico-medullary ratio, Thymocytes, Hassall's corpuscles, Histomorphometry*

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1. Introduction

The thymus acts as the principal lymphoid tissue involved in the development of a repertoire of T-lymphocytes with tolerance to self-antigen (Ruiz Pérez *et al.*, 2024). The parenchyma of this gland consists of two different types of anatomical structures known as the cortex and medulla, each composed of thymic epithelial cells in a 3D lattice (Han and Zúñiga-Pflücker, 2021). These T-lymphocyte precursors, which develop from the bone marrow, enter the gland at the cortico-medullary junction and after being exposed to the selection processes

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such as β and positive selections in the cortex, they move to the medulla for the last selection, known as the negative selection (Thapa and Farber, 2019).

Both thymic regions are responsible for distinct functions in immunity: the cortex is considered the primary site of thymocyte proliferation and positive selection, while the medulla participates in the process of negative selection and formation of regulatory T-cells. Hassall's corpuscles are found in the medulla; these concentric modifications of mature keratinized epithelial cells which produce thymic stromal lymphopoietin have been known to participate in the education of dendritic cells and CD4+CD25+ regulatory T-cells. Therefore, the ratio between cortex and medulla and the concentration of thymocytes in each of these areas reflect the functional activity of the thymus (Lancaster *et al.*, 2021; Watanabe *et al.*, 2005).

Since birth, there has been a continuous and programmed involution of the thymus gland. Microscopically, this is evident through the thinning and irregularity of the thymic cortex, decreasing cortico-medullary distinction, enlargement of the perivascular space, and fatty degeneration of the thymic parenchyma (Liang *et al.*, 2022). Involution starts at an earlier age than any other organ, hence its association with establishing the rate of aging of the T-cell system (Elyahu and Monsonego, 2021).

The involution has significant effects on the body. The decreased production of naive T-cells by the thymus results in the contraction of T-cell receptor repertoires, thus leading to aging and inflammation (immunosenescence and inflammaging), increased vulnerability to infections, low reactivity to vaccines, increase in autoimmune reactions and poor tumour immunity (Thomas *et al.*, 2020; Mittelbrunn and Kroemer, 2021). The medical importance of the thymus is shown by the finding that adult patients who undergo thymectomy have high mortality and incidence of cancers and autoimmune disorders (Kooshesh *et al.*, 2023).

From a mechanistic point of view, age-related atrophy is caused mainly by the thymic stromal environment and not just by any inherited defects in progenitor cells (Li *et al.*, 2024). One of the first things to happen in thymic aging is a decline in the levels of the forkhead-box transcription factor FOXN1 in thymic epithelial cells, thus leading to the destruction of the epithelial construction (Li *et al.*, 2023). Changes in the medullary epithelium also involve Hassall's corpuscles since their advanced state of differentiation and senescence-linked secretory properties are associated with a pro-inflammatory thymic microenvironment (Laan *et al.*, 2021; Wang *et al.*, 2019). In addition, endocrine signals such as gonadal and adrenal steroids are also known to contribute to this process in that changes in sex- and stress-steroid axes have been shown to affect the size of the thymus, cell density in the cortex, cortex-to-medulla ratio, and fat infiltration in a number of studies with rodent models of the condition (Chen *et al.*, 2026).

While there is sufficient description of the qualitative aspects of thymic involution, quantitative data on the changes in the cortico-medullary area ratio and the distribution of lymphocytes in these areas across the human life span are minimal and most of the mechanistic information comes from murine experiments. The current study was therefore set up to conduct a quantitative histomorphometric study of a human thymus from five different age groups, with a focus on the cortico-medullary area ratio, lymphocyte density, adipose tissue changes and Hassall corpuscle morphology, and to investigate the links of these structural parameters to chronological age.

2. Materials and methods

2.1. Study design and specimen selection

A retrospective, cross-sectional histomorphometric investigation was conducted using a sample group of 50 thymus tissues stored in the archives of the Department of Anatomy and Pathology. The tissues were collected from deceased individuals without any history of thymic, haematological, and immunological problems. Cases were stratified into five age groups:

- Group I (0–10 years)
- Group II (11–20 years)
- Group III (21–40 years)

- Group IV (41–60 years)
- Group V (61–80 years)

Cases with a history of primary or secondary immunodeficiency, hematolymphoid malignancies, mediastinal tumors, chronic debilitating illness, septicemia, prolonged corticosteroid or cytotoxic therapy, or autolyzed tissue unsuitable for morphometric analysis were excluded from the study.

2.2. Tissue processing and staining

Thymic samples were preserved using 10% neutral buffered formalin, routinely processed and embedded in paraffin. Tissue was sectioned into 4–5µm slices for H&E and Masson's trichrome staining in order to visualize the structure of the tissue and to identify connective tissues. The presence of adipose tissue was recognized by the characteristic appearance of adipocytes as unstained rounded vacuoles on H&E and confirmed through both techniques.

2.3. Immunohistochemistry

Immunohistochemistry was carried out on selected paraffin sections with antibodies against pan-cytokeratin (AE1/AE3), CD3 (thymocytes) and Ki-67 (cell-proliferation). Immunohistochemical staining was performed according to standard methods and sensors of antigen were prepared by the implementation of the antigen retrieval, staining with primary and secondary antibodies and visualization using diaminobenzidine (DAB) method. The proliferation index of the cortex Ki-67 has been represented as the percentage of Ki-67 positive nuclei amongst the total thymocytes counted in five high-power microscope fields.

2.4. Histomorphometric analysis

Digital images were acquired under standardized lighting conditions and analysed using ImageJ software. For each specimen, the corticomedullary (C:M) area ratio was calculated by dividing the total cortical area by the total medullary area across representative thymic lobules. Thymocyte density was measured separately in the cortex and medulla by counting nuclei, confirmed by CD3 staining, within calibrated microscopic fields and expressed as cells $\times 10^3/\text{mm}^2$. The proportions of parenchymal and adipose tissue were quantified using colour thresholding and point-counting stereology on whole-section image montages. Hassall's corpuscles were enumerated per mm^2 of medullary area, and their mean diameter was measured along the longest axis. All measurements were performed by observers blinded to age group.

2.5. Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD). Differences among the age groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Associations between histomorphometric variables and chronological age were evaluated using Pearson's correlation coefficient, with linear regression performed where appropriate. A two-tailed p-value of <0.05 was considered statistically significant. All statistical analyses were carried out using SPSS version 26.

3. Results

3.1. Sample characteristics

The demographic and gross characteristics of the 50 specimens are summarized in Table 1. The groups were comparable in sex distribution. Mean trimmed thymic tissue weight increased from infancy to a peak in the second decade and declined thereafter, consistent with the expected trajectory of thymic growth to puberty followed by progressive involution.

3.2. Gross and architectural changes

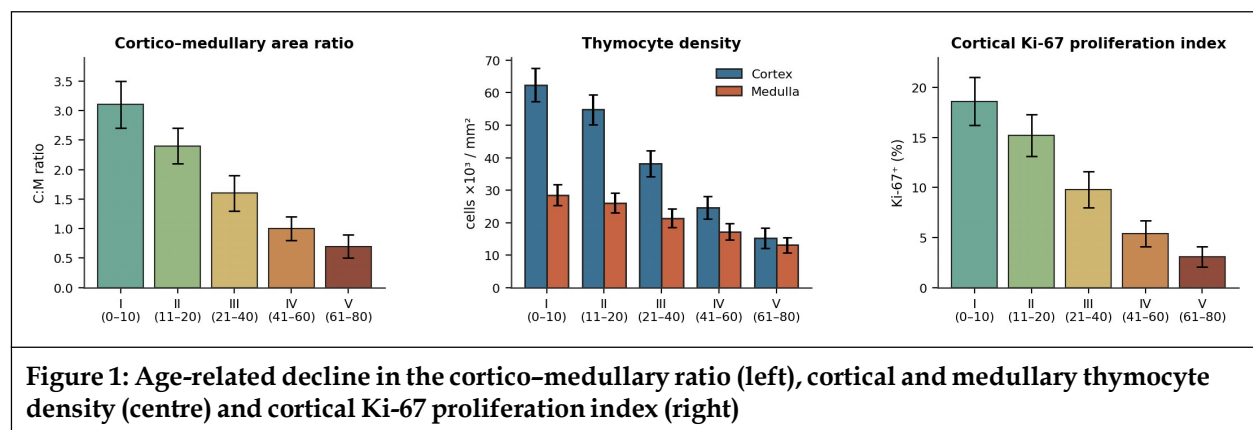
In the youngest group the thymus displayed a well-organised lobular architecture with a broad, deeply basophilic cortex densely populated by thymocytes, sharply demarcated from a paler, more cellular-sparse medulla. With advancing age the cortex progressively thinned, the cortico-medullary junction became increasingly indistinct, the medulla fragmented into isolated islands, and lobules were progressively separated and replaced by mature adipose tissue extending inward from the interlobular septa and subcapsular region.

Table 1: Demographic and gross characteristics of the study group

Group	Age range (y)	n	Mean age (y)	Male:Female	Mean thymic weight (g)
I	0–10	10	4.8 ± 3.1	6:4	22.4 ± 4.6
II	11–20	10	15.6 ± 2.8	6:4	28.7 ± 5.1
III	21–40	10	31.2 ± 5.7	5:5	20.3 ± 4.2
IV	41–60	10	51.4 ± 5.9	5:5	16.8 ± 3.8
V	61–80	10	70.3 ± 5.4	6:4	13.9 ± 3.5

3.3. Cortico-medullary ratio and lymphocyte distribution

The corticomedullary (C:M) area ratio declined significantly with age, decreasing from 3.1 ± 0.4 in Group I to 0.7 ± 0.2 in Group V ($p < 0.001$), indicating progressive thymic involution. The numbers of cortical thymocytes were significantly decreased 75-fold (from 62.4×10^3 to 15.3×10^3) and the number of medullary thymocytes were decreased 54-fold (from 28.5×10^3 to 13.1×10^3). As a result, the greatest age-related loss was in the cortex, with the cortical-to-medullary cell density ratio in the youngest group versus the oldest group being 2.19:1 versus 1.17:1, respectively. Likewise, the Ki-67 proliferation index was significantly reduced in the old group (Group V) compared to the young (Group I), and showed a progressive reduction of the thymic proliferative activity with increasing age (Figure 1).



3.4. Tissue composition and adipose involution

The quantitative analysis showed that there was an inverse correlation between the age and both the thymic parenchyma and adipose tissue. The parenchymal area reduced from 88.4% (Group I) to 19.8% (Group V) while the area of adipose tissue increased from 6.8 to 76.9 % in Group V ($p < 0.001$; Table 2). The functional epithelial-lymphoid tissue was evident only in small islands scattered throughout the adipose tissue throughout the sixth to eighth decades of life. The ratio of connective tissue and blood vessels, however, did not vary significantly among the age groups.

3.5. Hassall's corpuscles

A biphasic pattern was seen in Hassall's corpuscles with both increase and decrease in number and size among the age groups. The density of these was raised to 4.2/mm² in Group I and reached a peak in Group III of 6.8/mm², which is age-related decline of density in the thymic medulla in Group V is 4.5/mm². By contrast, the mean diameters continued to rise as a function of age from 28.4 μm to 71.3 μm. Older specimens frequently had Hassall's corpuscles which exhibited central keratinisation, cystic degeneration and calcification (Table 2).

3.6. Histopathological evaluation

Figure 2 depicts the complete configuration with respect to structural alteration in relation to age. Young thymic tissue has dense cellular cortex, a clearly stated cortico-medullary junction, compact medullary islands

Table 2: Thymic tissue composition and Hassall's corpuscle morphometry across age groups					
Parameter (per group)	I (0-10)	II (11-20)	III (21-40)	IV (41-60)	V (61-80)
Parenchymal area fraction (%)	88.4 ± 3.0	80.2 ± 3.8	60.1 ± 5.0	37.4 ± 5.5	19.8 ± 4.6
Adipose area fraction (%)	6.8 ± 2.1	14.5 ± 3.4	34.7 ± 5.2	58.3 ± 6.1	76.9 ± 5.8
Hassall's corpuscle density (mm ²)	4.2 ± 1.1	5.6 ± 1.3	6.8 ± 1.5	6.1 ± 1.4	4.5 ± 1.3
Hassall's corpuscle diameter (µm)	28.4 ± 5.1	34.2 ± 6.0	45.6 ± 7.2	58.9 ± 8.4	71.3 ± 9.6

that have a few Hassall's corpuscles, and a low amount of adipose tissue. On the other hand, elderly thymus has an obvious disappearance of cortex together with cortical thymocytes, loss of clear border between cortex and medulla, disintegration of medulla into individual islands with larger Hassall's corpuscles, and vast replacement of thymus parenchyma with adipocytes.

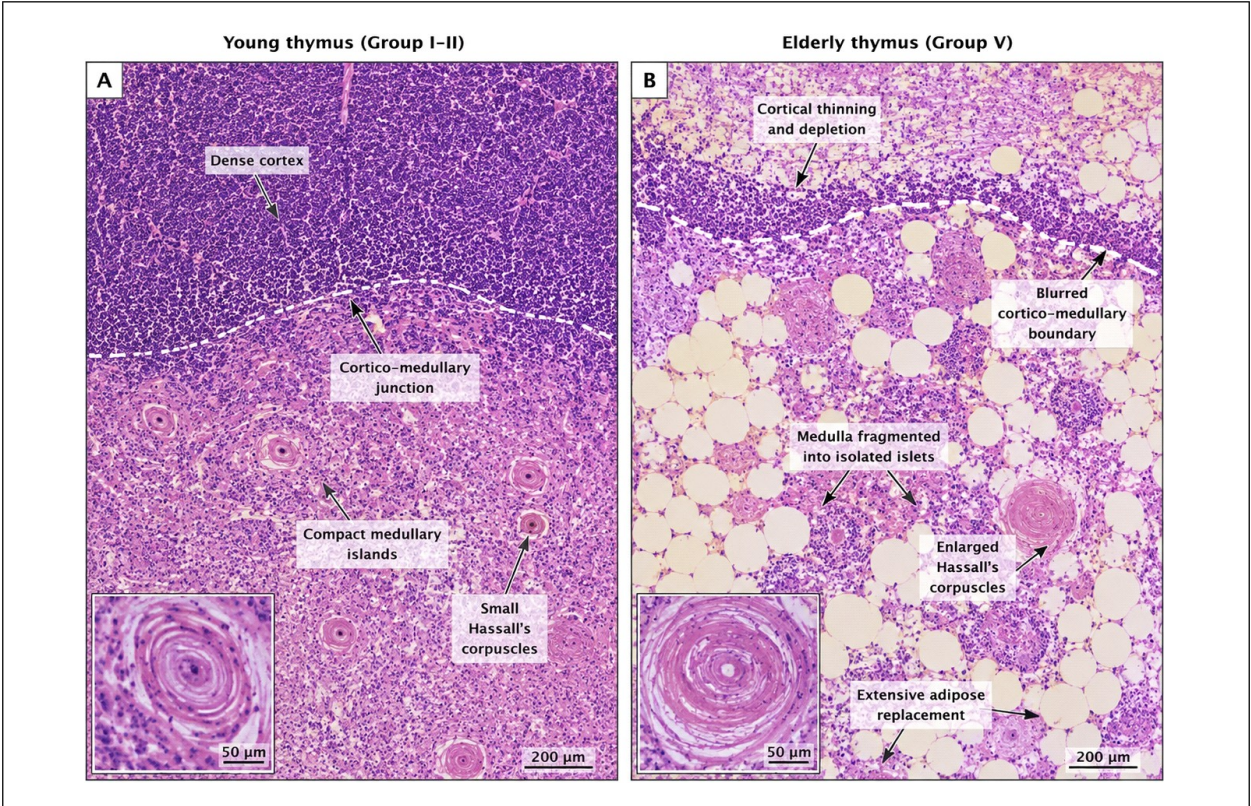


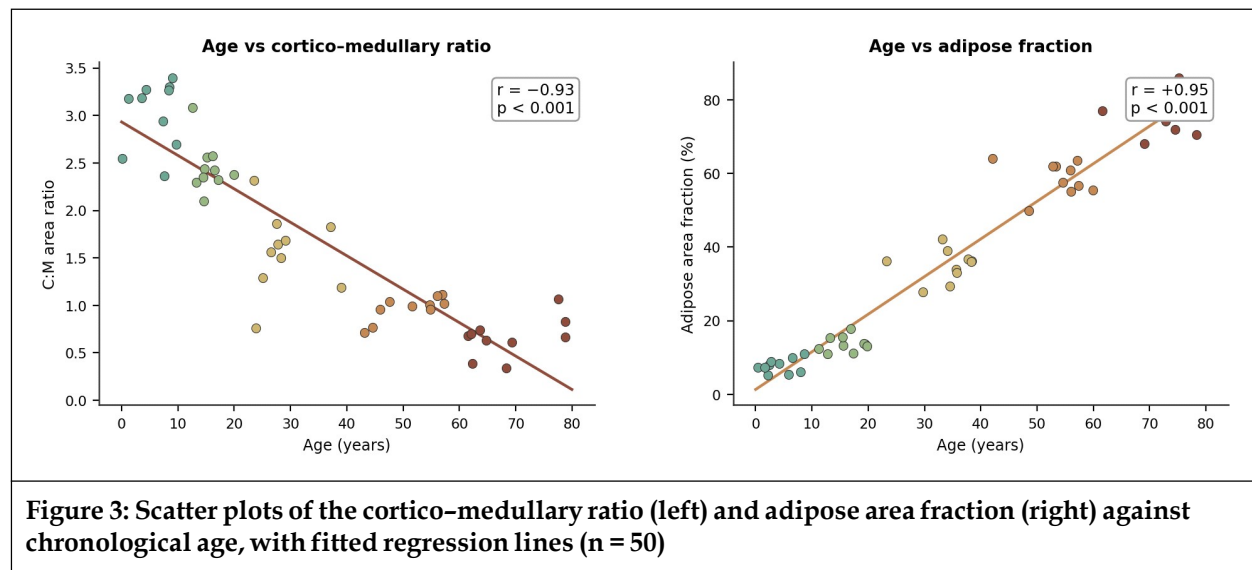
Figure 2: Representative histological comparison of thymic architecture in youth versus old age, contrasting cortical thymocyte density, cortico-medullary organisation, Hassall's corpuscle size and adipose involution

3.7. Correlation with chronological age

Throughout the entire pooled cohort (n = 50), chronological age was found to be strongly and negatively correlated with the cortico-medullary ratio (r = -0.93), cortical thymocyte density (r = -0.91), medullary thymocyte density (r = -0.86), cortical Ki-67 index (r = -0.89) and parenchymal area fraction (r = -0.94) and strongly and positively correlated with adipose area fraction (r = +0.95) and size of Hassall's corpuscles (r = +0.88); all correlations statistically significantly at p < 0.001 (Figure 3). These strong associations between chronological age and the cortico-medullary ratio and also adipose fraction indicate that these two parameters are very reliable markers of the thymus senescence process.

4. Discussion

The histomorphometric analysis reveals the quantitative aspects of the structural changes in the human thymus that occur throughout the course of human life. The most important findings were the continuous



collapse of the C-M ratio, disproportionate loss of the region of the cortex compared to the medulla, drastic decrease of the internal proliferation, as well as near complete transformation of the parenchyma into the adipose tissue by the 70s and 80s. The structural parameters have strong correlation with the time of life, and the C:M ratio and adipose tissue content turned out to be the most sensitive quantitative indicators of thymic ageing.

The decline in the C:M ratio corresponds to the already known qualitative feature of involution, the cortical atrophy with simultaneous cortico-medullary separation decay. The fact of the preferential depletion of cortical thymocytes as compared with the depletion of those in the medulla corresponds to the compartmental biology of the gland since it is the cortex where the expansion and positive selection of the progenitors occur, thus making it the most sensitive to the decrease of the supply and proliferation of the thymic progenitors during ageing. The decrease in the cortical Ki-67 index serves as direct confirmation of the decline of thymopoiesis.

The reciprocal increase in adipose tissue is a key element of the shrinking of the thymus and was the most important age-related variable in this investigation. The adipogenic transformation of thymic stroma is associated with the inception of atypical, mesenchyma-like thymic epithelial cells and with the elimination of the epithelial-supporting environment, two phenomena that are closely linked with persistent down-regulation of FOXN1. Our compositional data, showing adipose fractions exceeding 75% in the oldest group, provide a quantitative human counterpart to these predominantly experimental observations. Endocrine influences are also relevant: in rodent models, perturbation of gonadal and adrenal steroid axes bidirectionally alters cortical cellularity, the cortex-to-medulla ratio and the degree of adipose infiltration, echoing the structural axis quantified here and supporting a hormonally responsive component of involution.

The behaviour of Hassall's corpuscles merits particular comment. In the present series corpuscle diameter increased steadily with age, with older corpuscles frequently exhibiting keratinisation, cystic change and calcification, whereas corpuscle density peaked in early adulthood before declining. This biphasic pattern is consistent with the view that Hassall's corpuscles represent a terminal, post-AIRE stage of medullary epithelial differentiation; their progressive enlargement and acquisition of senescence-associated, pro-inflammatory features may contribute to the tonic inflammatory milieu of the ageing thymus. The eventual decline in corpuscle density in the oldest group most plausibly reflects fragmentation and attrition of the medulla itself rather than a primary reduction in corpuscle formation. It should be noted that the relationship between Hassall's corpuscle abundance and thymic activity remains debated, with some authors reporting that corpuscle number and size track thymic functional mass; our finding of enlarging corpuscles against a background of declining density is compatible with this nuanced picture.

Collectively, these structural changes have clear functional implications. The impairment of cortical thymocyte and epithelial architecture translates into the reduced ability of the gland to generate T-cells, with the relevant clinical significance of preserving any remaining thymic tissue appearing from the findings showing that thymectomy in adults correlates with the identifiable immunological and oncological

consequences in the long-term. The strong almost linear relationships established between the chronological age and C:M ratios as well as the proportion of the fat provide proof that, in fact, these quantitative histological parameters can serve as structural indicators of the process of aging in the thymus. At the same time, these parameters can become a morphological indicator against which all the new approaches of regeneration in the thymus are tested, including therapy based on FOXN1 and stimulation by epithelial growth factors or hormones, as well as engineered thymic organoids (Provin and Giraud, 2022).

5. Conclusion

Human thymic involution is characterised by a progressive, quantifiable collapse of the corticomedullary ratio, preferential depletion of cortical thymocytes, a marked decline in intrathymic proliferation, enlargement of Hassall's corpuscles and near-complete adipose replacement of functional parenchyma. Among the parameters examined, the corticomedullary area ratio and the adipose area fraction were the most strongly and consistently associated with chronological age, and together constitute robust structural indices of thymic ageing. These histomorphometric benchmarks may prove useful both for the morphological staging of thymic involution and as reference endpoints for future studies of thymic preservation and regeneration.

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