Semi-quantification of Myocardial Uptake of Bone-seeking Agents in Suspected Cardiac Amyloidosis

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Research Article

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Abstract

Introduction:

In recent years, bone scintigraphy has emerged as a key tool for non-invasive etiologic diagnosis of transthyretin (ATTR) cardiac amyloidosis (CA). The qualitative Perugini scoring system is currently the most widely-used method to evaluate myocardial uptake of bone-seeking agents. A semi-quantitative approach may, however, be more useful in clinical practice for both diagnosis and therapeutic follow-up. In this study we focused on a new semi-quantification method with the aim of improving the diagnostic accuracy of CA.

Material and Methods:

We retrospectively evaluated 8674 consecutive ^{99m}Tc-biphosphonate (HMDP or DPD) scintigraphies performed at the Nuclear Medicine Unit of the Azienda Ospedale-Università Padova between January 2012 and December 2016. The qualitative Perugini scoring system was compared with three recently proposed semi-quantitative indices (ratio of heart to thighs: RHT; ratio of lungs to thighs: RLT; ratio of femur to thighs: RFT) in 68 individuals presenting significant myocardial uptake (mean age 79 ± 7 years, range 62-100 years; female/male ratio 16/52). We took 349 consecutive bone scintigraphies qualitatively absent of any cardiac/pulmonary uptake as healthy controls (HC).

Results:

The RHT and RLT indices (for both males and females) were significantly higher in patients (visual Perugini scores from 1 to 3) than in HCs (p £0.0001). There were also statistically significant differences for RHT (in both sexes) in HCs vs patients with qualitative scores of 1 or >1 (with p ranging from £0.001 to £0.0001). Analysis of the ROC curves showed that RHT outperformed the other two indices (RLT and RFT) and was more sensitive and specific in both male and female groups. Furthermore, in the male population, RHT very accurately distinguished HCs and patients with scores of 1 (known to be less likely affected by ATTR) from patients with qualitative scores >1 (known to be more likely affected by ATTR) with an AUC of 99% (sensitivity: 95%; specificity: 97%).

Conclusion:

The proposed semi-quantitative RHT index can accurately distinguish between HCs and subjects with CA (Perugini scores from 1 to 3) in both females and males. Furthermore, RHT is able to predict with very high accuracy subjects in the male population more likely to be affected by ATTR (qualitative scores >1).

Introduction

Cardiac amyloidosis (CA) is an underdiagnosed cardiomyopathy characterized by diastolic dysfunction, restrictive hemodynamics, and heart failure resulting from the progressive deposition of insoluble amyloid fibrils in the myocardium(1). On the basis of the molecular structure of the amyloid deposits, it is possible to distinguish three main etiologies of CA, which together account for approximately 98% of clinical cases(2): primary light-chain (AL) amyloidosis, hereditary transthyretin (ATTRv; v = variant) and wild-type transthyretin (ATTRwt) amyloidosis. While the misfolding, accumulation and deposit of immunoglobulin light chains (monoclonal kappa or lambda) lead to AL CA(3), amyloid formation and deposition in transthyretin amyloidosis results from dissociation of the tetrameric transthyretin molecule (due either to a genetic mutation, ATTRv, or ageing in ATTRwt) and the misfolding of the resulting monomers into amyloid fibrils (4). The gold standard for CA diagnosis and subtyping is endomyocardial biopsy (EMB), and histopathological and immunohistochemical analysis(5), a procedure that has optimal sensitivity and specificity(6),(7), but has the disadvantages of high cost, invasiveness, and life-threatening complications in approximately 6% of patients(2).

A number of non-invasive diagnostic tools have so far been used in CA, including serum cardiac biomarkers (e.g. troponin T and NT-proBNP)(8), strain echocardiography(9), (10), and cardiac magnetic resonance imaging(11). However, none of these procedures provides accurate characterization of the amyloidogenic protein, which is fundamental for correct treatment planning (12).

Among the nuclear medicine molecular targets, conventional bone-seeking agents (high myocardial uptake is associated with ATTR CA subtypes, hereditary and wild-type), and amyloid PET (high myocardial uptake of 11C-PIB, 18F-Flutemetamol or 18F-Florbetaben has been associated with the AL CA subtype(13) and ATTRV30M amyloidosis (14)) have emerged as key procedures for the non-invasive etiologic diagnosis of cardiac amyloidosis (CA), allowing early instigation of therapy.

In the present study, we focused on bone-seeking agents because these radiopharmaceuticals have been more widely used in nuclear medicine to diagnose CA, and also because cardiac uptake may be incidentally found when a bone scan is performed for oncological/rheumatological indications.

Although the binding mechanism remains a matter of debate, three bone-seeking agents have been shown to be comparably effective in localizing cardiac amyloid deposits: pyrophosphate (^{99m}Tc-PYP), 3,3diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD), and more recently ^{99m}Tc-HMDP (hydroxymethylene diphosphonate) (15),(16),(17).

Reports of bone scans with increased myocardial ^{99m}Tc-PYP uptake in small cohorts of patients with CA date back to the early 1980s. However, the low intensity of the signal and false positive results in hypertensive, sarcoid and dilated cardiomyopathies has limited the use of this radiotracer(18),(19). A study comparing ^{99m}Tc-MDP with ^{99m}Tc-PYP in seven patients with cardiac amyloidosis reported sensitivities of 100% for the former versus 56% for the latter(20).

The possibility of distinguishing different CA subtypes was suggested by Perugini et al. on the basis of an initial study with 15 patients with ATTR and 10 patients with AL CA who underwent ^{99m}Tc-DPD scans. Radiotracer uptake was reported in all ATTR cases and in none of the AL patients(21). Although a second study with a higher number of patients (45 ATTR, 34 AL, and 15 control subjects) identified mild ^{99m}Tc-

DPD uptake in about one third of AL cases(22), a very high uptake of ^{99m}Tc-DPD is usually associated with the ATTR CA subtype(23).

Few studies have investigated the usefulness of ^{99m}Tc-HMDP for diagnosing ATTR CA. Cappelli et al. performed ^{99m}Tc-HMDP scans on 65 biopsy proven CA patients (26 AL, 39 ATTR) and reported no amyloid uptake in 92% of AL CA patients, and moderate/strong uptake in 93% of ATTR CA patients, thus showing ^{99m}Tc-PYP and ^{99m}Tc-DPD to have comparable accuracies(15). Abulizi et al. performed ^{99m}Tc-DPD and 99mTc-HMDP scans (of six biopsy proven ATTR CA patients) and reported similar heart/mediastinum ratio values for both tracers(17). More recently, Musumeci et al. showed(24) both ^{99m}Tc-DPD and ^{99m} HMDP to have low levels of accuracy with regard to Phe64Leu mutation-related transthyretin CA.

Qualitative visual assessment of myocardial uptake, as proposed by Perugini et al., is the most widely-adopted method to evaluate CA(21). A more recent study by Hutt et al. demonstrated cardiac uptake on SPET/CT scans in seven CA patients (5 AL with median Grade 1 intensity, and 2 ATTRv) that would otherwise have been missed on planar image evaluation by two experienced nuclear medicine specialists(25). These authors therefore proposed revising the Perugini visual scoring system by taking into account SPET/CT and soft-tissue uptake.

Due to the frequent need for specific cut-off values both for diagnosis and for therapeutic management and follow-up, a semi-quantitative approach may be more useful than a purely qualitative approach in clinical practice.

Therefore, in this study we focused on a new semi-quantification method using bone-seeking agents (and planar whole-body acquisition) with the aim of developing a simple method that would improve the diagnostic accuracy of CA.

Materials And Methods

8674 consecutive ^{99m}Tc-biphosphonate (HMDP or DPD) scintigraphies performed mainly for oncological staging or rheumatologic diseases at the Nuclear Medicine Unit of the Azienda Ospedale-Università Padova between January 2012 and December 2016 were retrospectively evaluated in order to identify patients showing cardiac uptake. Patients aged <18 years were excluded from the study. The local ethical committee approved the retrospective study with reference number 24n/AO/20.

All bone scans were performed 2.5 to 4 hours after intravenous administration of 600-800 MBq of ^{99m}Tc-biphosphonates, depending on the patient's weight and age, using either a dual-headed gamma camera equipped with low-energy, high-resolution (HR) parallel hole collimators (InfiniaTM, GE Healthcare) or a triple-headed gamma camera with low-energy, ultra-high resolution (UHR) parallel hole collimators (IrixTM, Philips Medical System). Delayed (2.5 - 4 h) whole body images in anterior and posterior views were acquired using a predefined time frame (20-25 min, at a scanning speed of 15 - 18 cm/min) and a matrix size of 1024 x 256.

When myocardial uptake was observed qualitatively, the data were processed as follows:

1. ***Qualitative analysis*:** each patient was classified using the standard qualitative visual score described by Perugini et al.(*21*) (SPET data were available for only a minority of patients and were excluded):

- score 0, no cardiac uptake and normal bone uptake
- score 1, mild cardiac uptake that is less than bone uptake
- score 2, moderate cardiac uptake accompanied by attenuated bone uptake
- score 3, strong cardiac uptake with mild/absent bone uptake

We gave a ‘doubtful’ classification to cases where cardiac uptake did not properly fit the Perugini qualitative score, i.e. uptake was too mild or was not clearly attributable to the heart.

2. ***Semi-quantitative analysis:*** *c*ardiac uptake was then evaluated using a semi-quantitative method, which covered:

- *Heart uptake:* regions of interest (ROIs) were manually drawn on the anterior view as accurately as possible around the heart uptake, excluding ribs and sternum, and on the medial portion of the soft tissues (therefore mainly adipose tissue) of both the mid-thighs (Figure 1), excluding vessel uptake. The heart to thigh ratio (RHT) was then computed as (heart average counts – mid-thigh average counts)/mid-thigh average counts.
- *Pulmonary uptake:* since most patients with bone tracer cardiac uptake also showed particularly increased pulmonary uptake, the lungs were also semi-quantitatively assessed. A ROI in the right lung, including part of the mediastinum and excluding ribs and sternum, was drawn as accurately as possible. The semi-quantitative lung to thigh ratio (RLT) was then computed as (pulmonary average counts - mid-thigh average counts)/mid-thigh average counts.
- *Bone uptake:* since patients with intense cardiac uptake often showed an apparent reduction in bone uptake, we also defined ROIs on both femoral diaphyses (Fig 1) of each patient with cardiac uptake. The semi-quantitative femur to thigh ratio (RFT) was then computed as (femoral average counts - mid-thigh average counts)/mid-thigh average counts.

Dataset of normal subjects: to obtain a consistent normal reference database (healthy controls: HC) from which to determine cut-offs, of the 8677 consecutive bone scintigraphies examined (excluding positive and doubtful cases) we selected 349 where no cardiac/pulmonary uptake was qualitatively observed and where the subjects were aged ^{3}50 years. RHT, RLT, and RFT were calculated for each HC. The relatively high number of HC individuals compared with the patient population was due to the need to split the HC cohort by gender (males vs females), and to verify whether the reference values varied with age/sex. For this purpose, we grouped HCs as follows: females aged 50-64 years (healthy young females: HYF), females ³65 years (healthy elderly females: HEF), males aged from 50-64 years (healthy young males: HYM), males ³65 years (healthy elderly males: HEM).

Statistical analysis

The population was divided into three groups: healthy controls (HC), patients, and doubtful results.

An unpaired t-test was used to compare the three ratios - RHT, RHT, and RFT - among the three groups. Statistical significance was set at <0.05.

Receiver operating characteristic (ROC) curves were constructed and the Youden's index calculated to determine the cut-off value that best discriminated between the HC and patient groups. The cut-offs were then used to classify the doubtful group.

We also determined the cut-off values for discriminating the HCs and patients with a score of 1 versus the patients with scores of 2 and 3.

Statistical analyses were performed using the R software(*25*) and the pROC package(*26*).

Results

The averages ± standard deviations of the demographic data and ratios values are summarized in Table 1. The HC population comprised 349 subjects with a mean age of 66 ± 10 years (range 50-90 years; 166 females, 183 males). The patient population comprised 68 individuals with a mean age of 79 ± 7 years (range 62-100 years; 16 females, 52 males), and there were 27 patients classified as doubtful (mean age: 70 ± 10 years, range: 49-86 years; 11 females, 16 males).

Of the patient population (n=68), 43 (63%) had a Perugini score of 1, 18 (27%) a score of 2, and 7 (10%) a score of 3.

HC analysis:

Analysis by the Kruskal-Wallis test of the variability in the ratios according to age and sex in the HC cohort showed no significant differences between the two male HC classes (HYM vs HEM) in any of the three ratios ($p > 0.80$), while significant differences between the two female HC classes (HYF vs HEF) were found in RHT ($p < 0.00001$), and in RLT and RFT ($p < 0.01$). Significant differences were also found between males and female in all three ratios (HYF vs HYM: $p < 0.00001$; HEF vs HEM: $p < 0.01$). Therefore, in order to accurately match the average ages of the female populations (pathologic and HC), we excluded female HCs under 65. The new HC population comprised 89 females, 183 males.

To determine whether the chosen reference area (mean of both thighs) was responsible for the observed differences in gender and age, the mean values of the reference areas in HYF, HEF, HYM, and HEM were calculated and compared. A Kruskal-Wallis test revealed statistically significant differences in the reference areas between HYF and HEF ($p < 0.001$), between HYF and HYM ($p < 0.01$), and between HYF and HEM ($p < 0.0001$), but there were no differences between HYM and HEM ($p = 0.12$).

Overall analysis:

The results of the statistical comparison by Kruskal-Wallis test of the three computed ratios (RHT, RHT, and RFT) in male vs female populations are shown in Figures 2 to 7.

Regarding the female group:

- RHT and RLT were significantly higher in patients than in HCs (p-values £0001) (see Figures 2 and 3). Both indices also differed significantly in patients with qualitative scores of 1 or >1 versus HCs (p ranging from £0.01 to £0.0001, with the lower levels of significance for RLT).
- Only two statistically significant (p £05) differences in RFT were found: between HC and doubtful patients, and between HC and patients with scores >1 (see Figure 4).
- doubtful patients differed significantly (p £05) from HCs in the RHT and RFT indices but not in RLT.

Regarding the male group:

- The RHT and RLT indices in patients were significantly higher than in HCs (p-values £0001) (see Figures 5 and 6). RHT was also significantly higher in patients with qualitative scores of 1 or >1 (p £0.0001) than in HCs.
- Only two statistically significant differences in RFT were found: between HCs and patients (visual scores from 1 to 3; p £01), and between HCs and patients with scores >1 (p £0.05) (see Figure 7).
- doubtful patients differed significantly (p £05) from HCs in the RHT index but not in RLT or RFT.

Cut-offs by ROC curves: ROC curves and the corresponding values (sensitivity, specificity, cut-offs and AUC) for the three ratios (RHT, RHT, and RFT) in the HC and patient groups were computed separately for female and male populations. The results are shown in Table 2 (females) and Table 4 (males). The area under the curve (AUC) of RHT in terms of significantly detecting cardiac uptake (scored visually from 1 to 3) of the bone tracer was 0.96 (95% CI 0.90–1,00) in females, and 0.87 (95% CI 0.80–0.94) in males. The best cut-off value of RHT for females was 3.260, which provided a sensitivity of 94%, and a specificity of 87%. For males, the best cut-off value of RHT was 2.965, which provided a sensitivity of 81% and a specificity of 88%. Cut-off values were used to re-classify female (Table 3) and male (Table 5) individuals previously qualitatively classified as doubtful. We were thus able to reclassify as patients 6/11 (54.5%) females and 5/16 (31.25%) males using RHT, 2/11 (18%) females and 2/16 (12.5%) males using RLT, and 2/11 (18%) females and 6/16 (38%) males using RFT.

To ascertain whether the proposed indices were also able to reliably distinguish HCs and patients with a score of 1 (known to be less likely affected by ATTR) from patients with qualitative scores >1 (known to be more likely affected by ATTR), we grouped the male population on the basis of their visual Perugini (P) scores (HC+P1 vs P2+P3). We were not able to do the same for the female population due to the small sample size. We used the cut-off values (shown in Table 7) to re-classify as ATTR 1/16 (6.25%) doubtful male subjects using RHT, and 2/16 (12.5%) using RLT.

Discussion

In the present study, new semi-quantitative methods for the assessment of myocardial uptake of bone-seeking agents were retrospectively tested in a large cohort of patients undergoing bone scintigraphy for different clinical reasons. The main result is that the proposed RHT semi-quantitative index was able to distinguish between healthy controls and patients with significant cardiac uptake, i.e. with Perugini visual scores from 1 to 3, with an AUC of 96% (sensitivity: 94%, specificity: 87%) in the female population, and an AUC of 87% (sensitivity: 81%, specificity: 88%) in the male population. Using RHT, we were able to re-classify as positive a number of subjects - 6/11 (54.5%) females, and 5/16 (31.2%) males - previously qualitatively classified as doubtful. The proposed RHT metric may, therefore, help improve qualitative interpretation, especially in doubtful cases, and could be used in longitudinal evaluations where qualitative analysis is well known to be limited.

More interestingly, in the male population RHT was also able to reliably distinguish HCs and patients with a score of 1 (known to be less likely affected by ATTR) from patients with qualitative scores >1 (known to be more likely affected by ATTR) with an AUC of 99% (sensitivity: 95%, specificity: 97%), allowing us to qualitatively reclassify doubtful patients as probable ATTR. This is crucial, given the new treatments approved for ATTR(*1*), as the more objective semi-quantitative criteria may improve disease management by prompting genetic testing leading to early initiation of appropriate therapeutic treatments.

As for the majority of ratios used in nuclear medicine, the chosen reference area should ideally be free from the disease of interest (in our case CA). In the present paper, the mean value of the uptake by the medial portion of both thighs (excluding vessel uptake, and therefore mainly referrable to adipose tissue) was used as the reference area as qualitative visual assessment found the lung (or contralateral mediastinum), bone and muscles to be sites of dubious uptake. A significant difference in the mean values of the reference area between “young” and “old” females, and between genders was observed, which were lower in the young female group compared with the other groups. This might be due to the different lipidic contents (in the medial thigh soft tissue) of young females compared with older female and males, probably because of the modulatory role of sex steroids. The HEF group (i.e. excluding the younger female subjects) was therefore taken as the normal population because all the female pathologic subjects (except 1 doubtful patient) were over 65 years of age. No statistically significant age-related differences in the reference area were observed in the male population.

Although the proposed RLT metric differed significantly between HCs and subjects with significant cardiac uptake (Figures 3 and 6), it performed worse than RHT, presenting an AUC of 81% (sensitivity: 88%, specificity: 66%) in the female population, and an AUC of 67% (sensitivity: 33%, specificity: 95%) in the male population. Despite its likely unsuitability for clinical use in diagnosing CA, the RLT index and the comparisons with HCs confirm the visual impression of increased uptake by the lungs of patients with CA. It therefore seems inappropriate to use a heart-to-contralateral side (as the reference area) ratio as an index for CA.

Concerning the RFT metric, although a number of mildly significant differences were found in HCs vs patients (visual scores from 1 to 3), doubtful subjects, or patients with scores >1 (Figures 4 and 7), ROC curve analysis revealed too low a sensitivity (50% for the female group, 38% for the male group) for RFT to be considered a useful index for distinguishing HC from CA subjects. A similarly low sensitivity (57%; Table 6) was also found also in the male population when HCs and subjects with a visual score of 1 were grouped against subjects with visual scores >1. Nevertheless, we consider this analysis to be useful since it shows that "attenuated bone uptake" or "mild/absent bone uptake", as indicated by the Perugini scores refer mainly to how the images are normalized rather than to any real reduction in bone uptake.

Limitations

The bias of having mixed gamma-cameras/collimators, and protocols that were not identical (although very similar) is a consequence of the retrospective nature of the study. It may, on the other hand, simulate the heterogeneity of the real-world diagnostic setting. This bias may have been partially mitigated by the fact that ratios were used to calculate the indices.

Although no pathologic confirmation was obtained in this study, retrospective examination of their medical records revealed that 12/25 patients with visual scores of 2 or 3 presented with heart failure and concentric hypertrophy of the left ventricle, supporting the hypothesis that this was an ATTR population. We have no follow-up data for the remaining 13/25. Nor do we have any genetic testing, cardiac MR, strain echocardiography or pathology data for the retrospectively identified population.

Further studies are also desirable to determine whether the proposed RHT index is able to depict variations in heart uptake over time.

Conclusion

The proposed semi-quantitative RHT index is able to accurately distinguish HCs from subjects with significant cardiac uptake of bone-seeking agents (with Perugini scores from 1 to 3) in both females and males. Furthermore, in the male population, RHT distinguished with a high level of accuracy HCs and patients with a score of 1 (known to be less likely affected by ATTR) from patients with qualitative scores >1 (known to be more likely affected by ATTR) with an AUC of 99% (sensitivity: 95%; specificity: 97%).

Declarations

Compliance with Ethical Standards:

Funding: This research received no external funding.

Disclosure of potential conflicts of interest: All the authors declare no conflict of interest.
Research involving human participants and/or animals: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This was a single-center, retrospective, observational study conducted after formal approval by our local Ethics Committee (protocol number: reference number 24n/AO/20).
Informed consent: All patients gave written informed consent before undergoing the bone scintigraphy.

Author Contributions: Conceptualization, C.C., C.B., A.S. and D.C.; methodology, C.C., C.B., S.G., A.S. and D.C.; software, S.G. and C.C.; formal analysis, S.G.; C.C.; data curation, P.A., P.Z., C.C. and D.C.; writing—original draft preparation, R.V., A.C. and D.C.; writing—review and editing, C.C., C.B., P.Z., R.V., A.S., and D.C.; project administration, D.C. All authors read and agreed to the published version of the manuscript.

KEY POINTS:
QUESTION: Is it possible to easily semi-quantify the uptake of bone-seeking agents to improve the diagnostic accuracy of cardiac amyloidosis (CA) ?

PERTINENT FINDINGS:

A patient population comprising 68 individuals with significant myocardial uptake of ^{99m}Tc-biphosphonate (HMDP or DPD) and an HC population of 349 subjects have been used to test the proposed semi-quantification approach that is able, with a high level of accuracy (AUC of 99%), to distinguish HCs from patients likely affected by ATTR amyloidosis.

IMPLICATIONS FOR PATIENT CARE:

Given the new treatments approved for ATTR amyloidosis, the proposed objective semi-quantitative criteria may improve disease management by prompting genetic testing leading to early initiation of appropriate therapeutic treatments.

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Tables

Table 1 Demographic data and ratio values for the whole population grouped by males and females. HC: healthy controls; D: doubtful patients; P score = 1-2-3: groups of patients with Perugini scores of 1-2-3, respectively; P-total: all patients with scores of 1, 2, and 3.

	HC	D	P score = 1	P score = 2	P score = 3	P-total
FEMALES						
Number	89	11	12	3	1	16
Age (years)	73 ± 6	71 ± 8	78 ± 7	81 ± 5	83	79 ± 6
Age range (years)	65 - 90	49 - 79	66 - 88	77 - 86	83	66 - 88
RHT	2.52 ± 0.75	3.30 ± 0.93	5.39 ± 1.92	10.79 ± 4.79	21.91	7.43 ± 5.03
RLT	2.58 ± 0.85	2.62 ± 0.71	3.83 ± 1.56	4.20 ± 0.83	5.73	4.02 ± 1.45
RFT	2.74 ± 1.17	2.13 ± 0.61	2.60 ± 1.12	1.70 ± 0.11	1.88	2.39 ± 1.03
MALES						
Number	183	16	31	15	6	52
Age (years)	68 ± 9	68 ± 11	78 ± 8	80 ± 6	82 ± 5	79 ± 7
Age range (years)	50 - 86	50 - 86	62 - 100	69 - 86	72 - 89	62 - 100
RHT	2.28 ± 0.57	2.80 ± 0.83	3.55 ± 1.39	8.39 ± 3.55	12.34 ± 6.14	5.96 ± 4.25
RLT	2.26 ± 0.60	2.35 ± 0.92	2.44 ± 0.68	3.53 ± 0.94	4.92 ± 2.20	3.04 ± 1.33
RFT	2.31 ± 1.77	1.72 ± 0.70	1.87 ± 0.73	1.91 ± 1.02	1.35 ± 1.12	1.82 ± 0.84

Table 2 Sensitivity, specificity, cut-offs, and AUC values (95% CI) for the three ratios in the female population.

	Females (n=105)			
	HC (n=89), P (n=16)			
	Sensitivity (95% CI)	Specificity (95% CI)	Cut-off	AUC (95% CI)
RHT	0.94 (0.81 ; 1.00)	0.87 (0.79 ; 0.93)	≥ 3.260	0.96 (0.90 ; 1.00)
RLT	0.88 (0.69 ; 1.00)	0.66 (0.57 ; 0.76)	≥ 2.825	0.81 (0.68 ; 0.95)
RFT	0.50 (0.25 ; 0.75)	0.76 (0.67 ; 0.76)	< 1.890	0.59 (0.43 ; 0.76)

Table 3 Re-classification of the qualitatively doubtful female patients on the basis of the semi-quantitative cut-off values

Doubtful	Females (n=11)
	Cut-off
RHT	n = 6 (54.54%) ≥ 3.260
RLT	n = 2 (18.18%) ≥ 2.825
RFT	n = 2 (18.18%) < 1.890

Table 4 Sensitivity, specificity, cut-offs and AUC values (95% CI) for the three ratios in the male population.

	Males (n=235)			
	HC (n=183), P (n=52)			
	Sensitivity (95% CI)	Specificity (95% CI)	Cut-off	AUC (95% CI)
RHT	0.81 (0.69 ; 0.90)	0.88 (0.83 ; 0.93)	>2.965	0.87 (0.80 ; 0.94)
RLT	0.33 (0.19 ; 0.46)	0.95 (0.92 ; 0.98)	>3.290	0.67 (0.59 ; 0.76)
RFT	0.38 (0.25 ; 0.52)	0.86 (0.81 ; 0.91)	<1.305	0.62 (0.53 ; 0.71)

Table 5 Re-classification of qualitatively doubtful male patients on the basis of the semi-quantitative cut-off values.

Doubtful	Males (n=16)
	Cut-off
RHT	n = 5 (31.25%) > 2.965
RLT	n = 2 (12.5%) > 3.290
RFT	n = 6 (37.5%) < 1.305

Table 6 Sensitivity, specificity, cut-offs and AUC values (95% CI) for the three ratios in the male sub-group computed on the basis of patients with a qualitative score of 1 + HC versus patients with qualitative scores >1 (2 and 3).

	Males (n=235)			
	HC + Score = 1 (n=214) vs Score >1 (n=21)			
	Sensitivity (95% CI)	Specificity (95% CI)	Cut-off	AUC (95% CI)
RHT	0.95 (0.86 ; 1.00)	0.97 (0.95 ; 0.99)	≥ 4.69	0.99 (0.98 ; 1.00)
RLT	0.66 (0.47 ; 0.86)	0.94 (0.91 ; 0.97)	≥ 3.29	0.86 (0.76 ; 0.96)
RFT	0.57 (0.38 ; 0.76)	0.75 (0.69 ; 0.81)	< 1.515	0.63 (0.48 ; 0.78)

Table 7 Re-classification of qualitatively doubtful male patients using the semi-quantitative cut-off values computed on the basis of patients with a qualitative score of 1 versus patients with scores >1 (2 and 3).

Doubtful	Males (n=16)
	Cut-off
RHT	n = 1 (6.25%) ≥ 4.69
RLT	n = 2 (12.5%) ≥ 3.29
RFT	n = 9 (56.25%) < 1.515

Figures

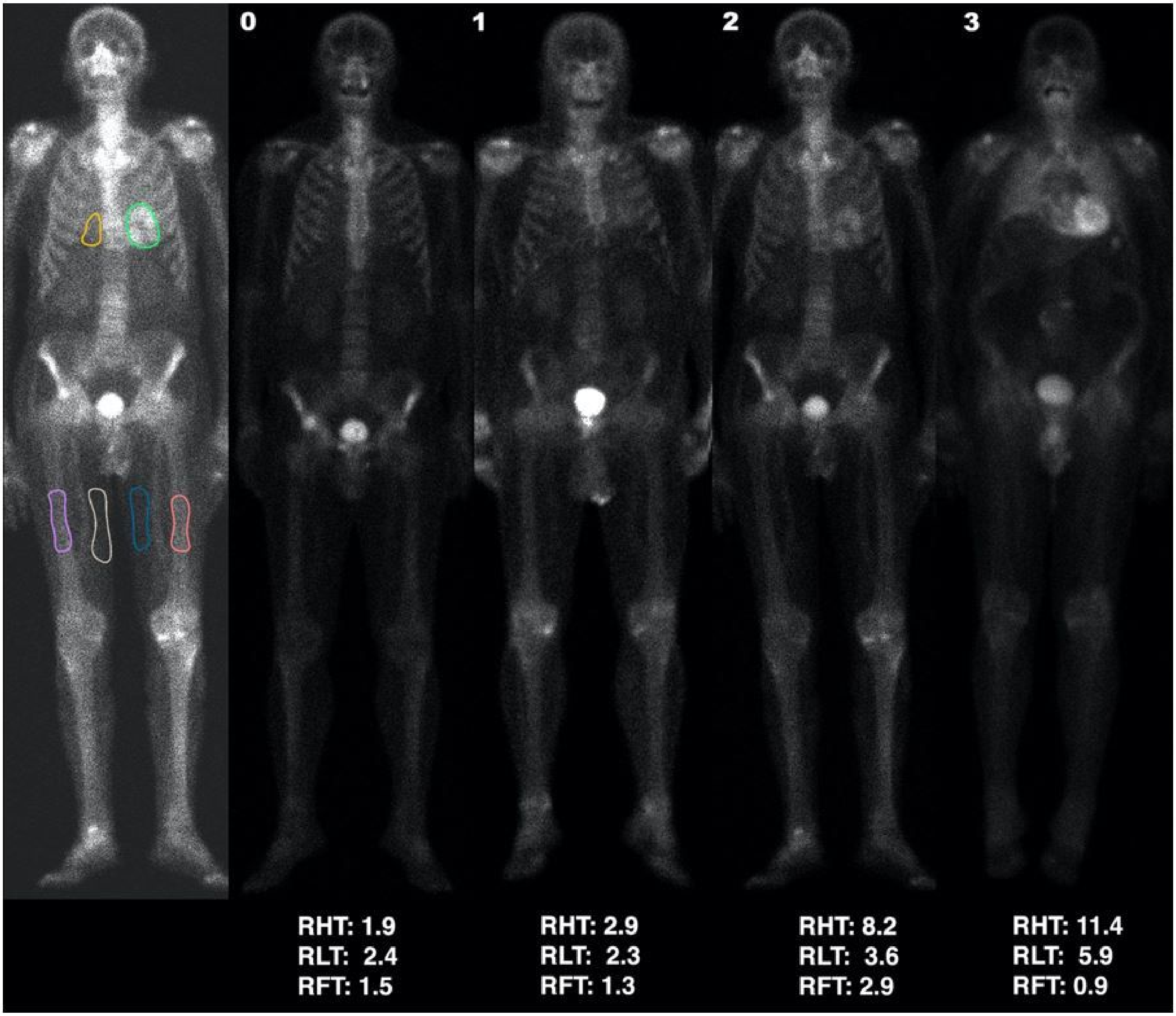

Figure 1

First column: ROIs (heart: green; lung: yellow; thighs: blue and white; bone: pink and orange) used to calculate the RHT, RLT, and RFT indices (presented under each patient). Examples are of an HC (score 0; second column) and patients with scores of 1, 2, and 3 (third, fourth and fifth columns, respectively).

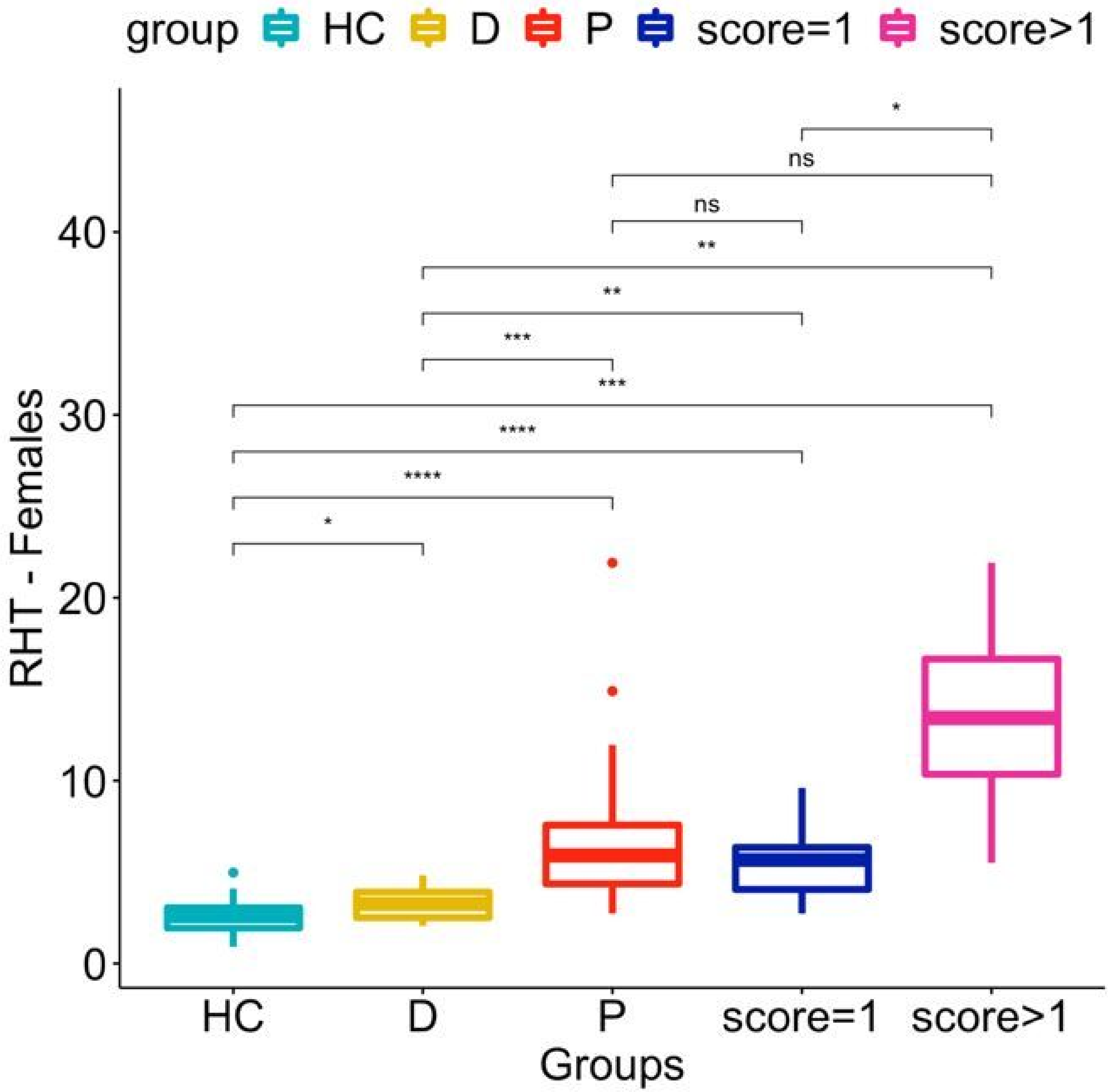

Figure 2

Distribution of RHT values for healthy controls (HC), doubtful cases (D), patients (P), patients with score = 1, and patients with scores >1 across the female population. ns: p > 0.05 ; * p ≤0.05; ** p ≤0.01; *** p ≤0.001; **** p ≤0.0001.

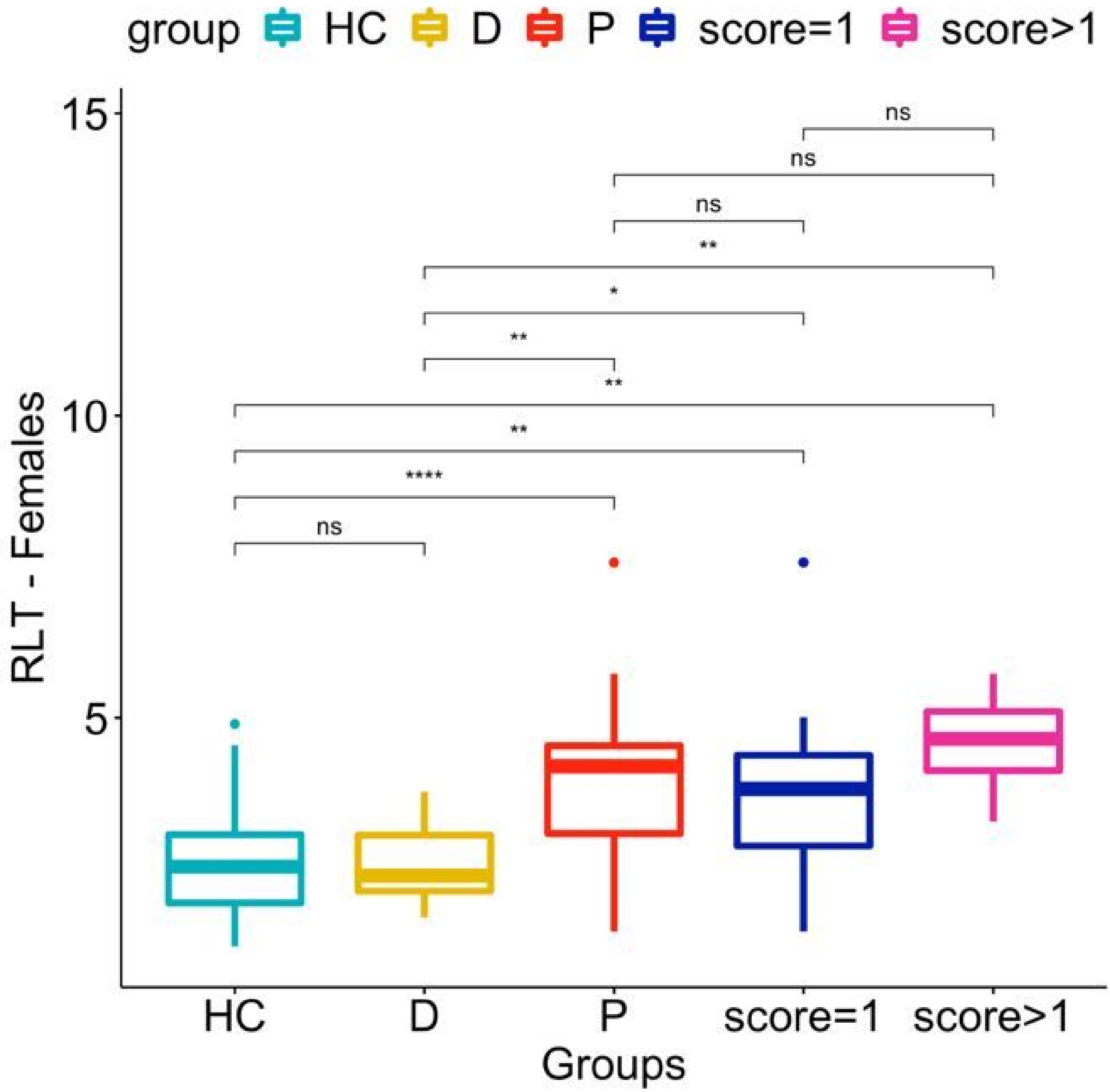

Figure 3

Distribution of RLT values for healthy controls (HC), doubtful cases (D), patients (P), patients with score = 1, and patients with scores >1 across the female population. ns: p > 0.05 ; * p ≤0.05; ** p ≤0.01; *** p ≤0.001; **** p ≤0.0001.

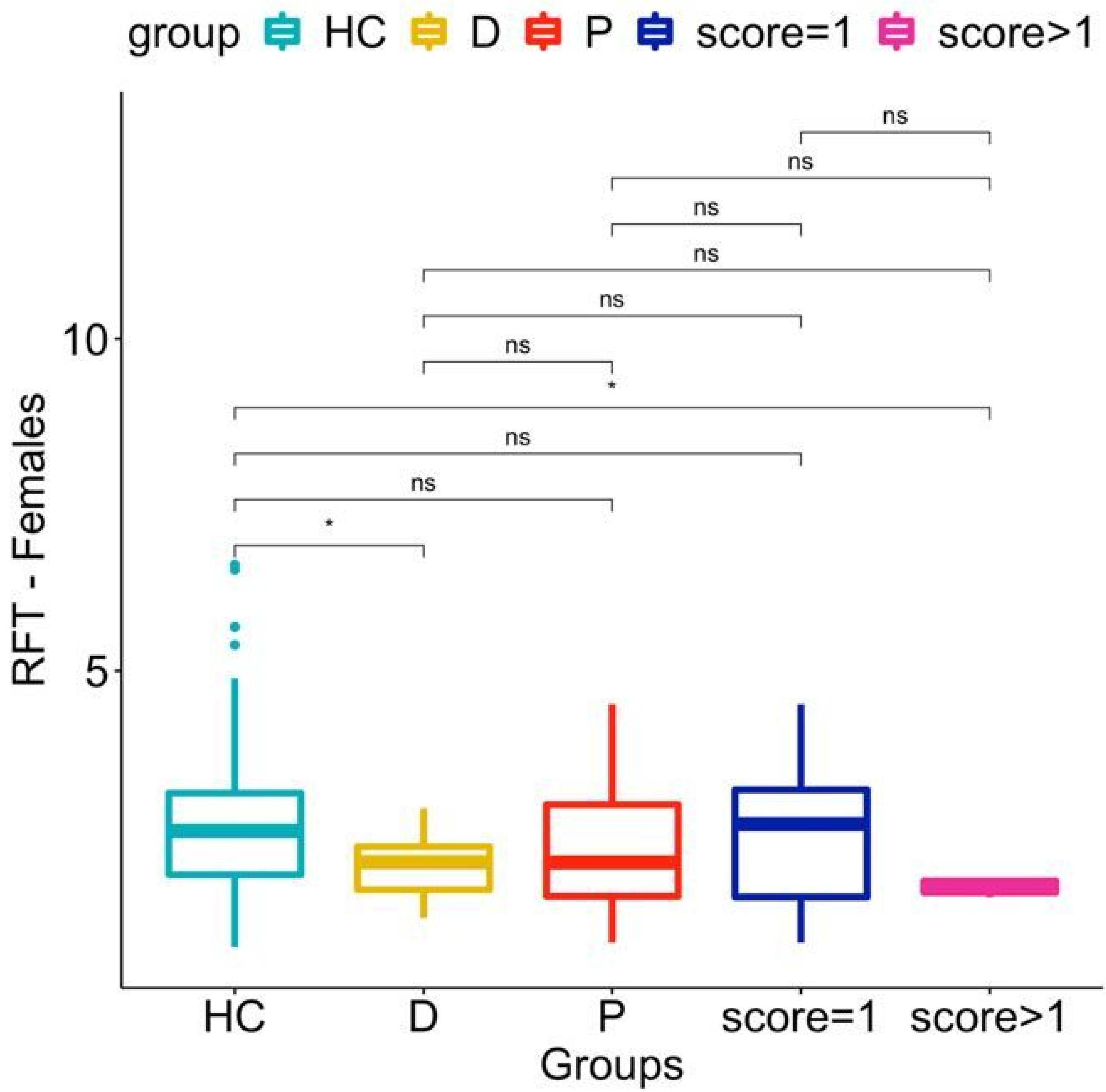

Figure 4

Distribution of RFT values for healthy controls (HC), doubtful cases (D), patients (P), patients with score = 1, and patients with scores >1 across the female population. ns: p > 0.05 ; * p ≤0.05; ** p ≤0.01; *** p ≤0.001; **** p ≤0.000

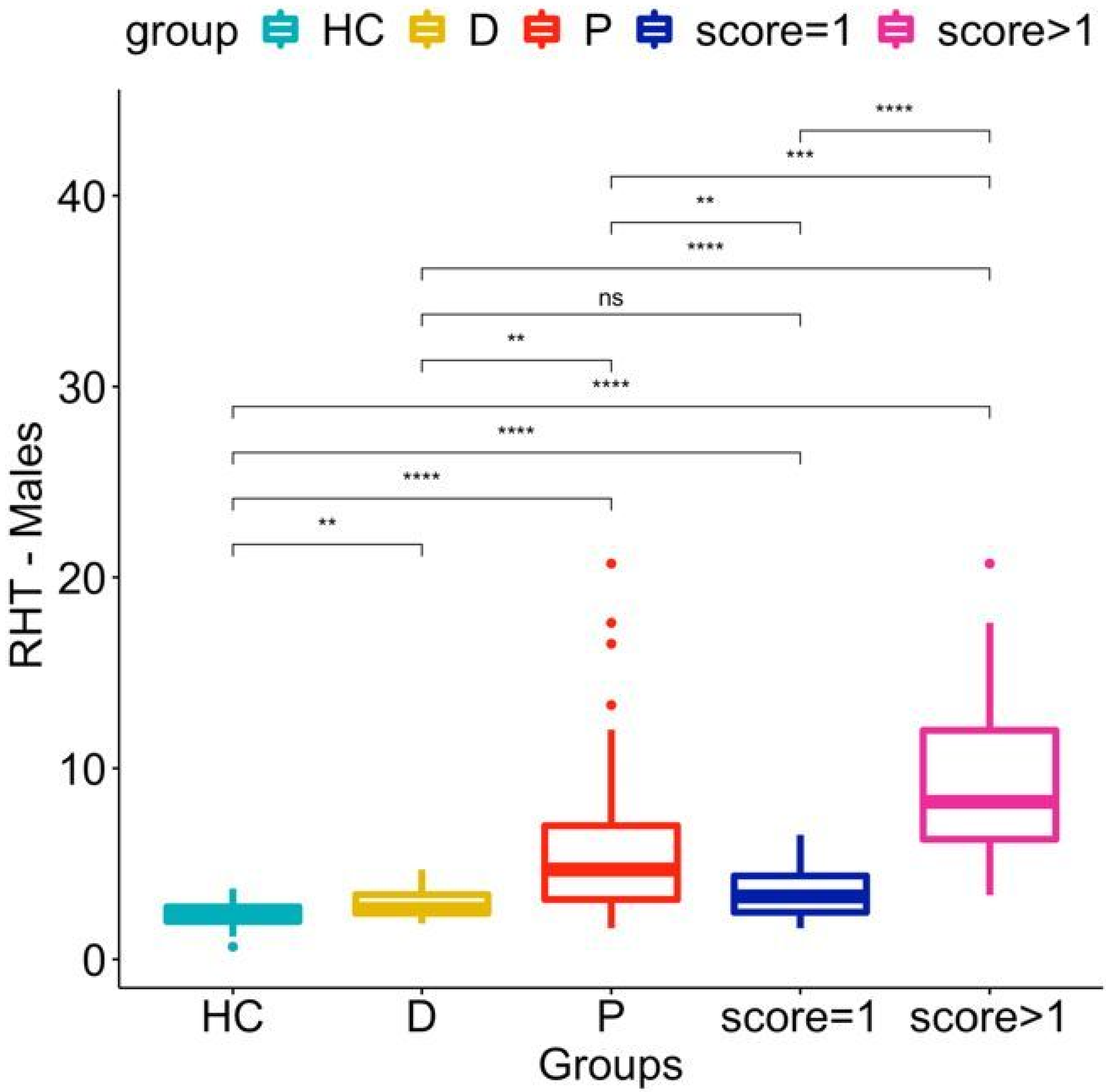

Figure 5

Distribution of RHT values for healthy controls (HC), doubtful cases (D), patients (P), patients with score = 1, and patients with scores >1 across the male population. ns: p > 0.05 ; * p ≤0.05; ** p ≤0.01; *** p ≤0.001; **** p ≤0.0001.

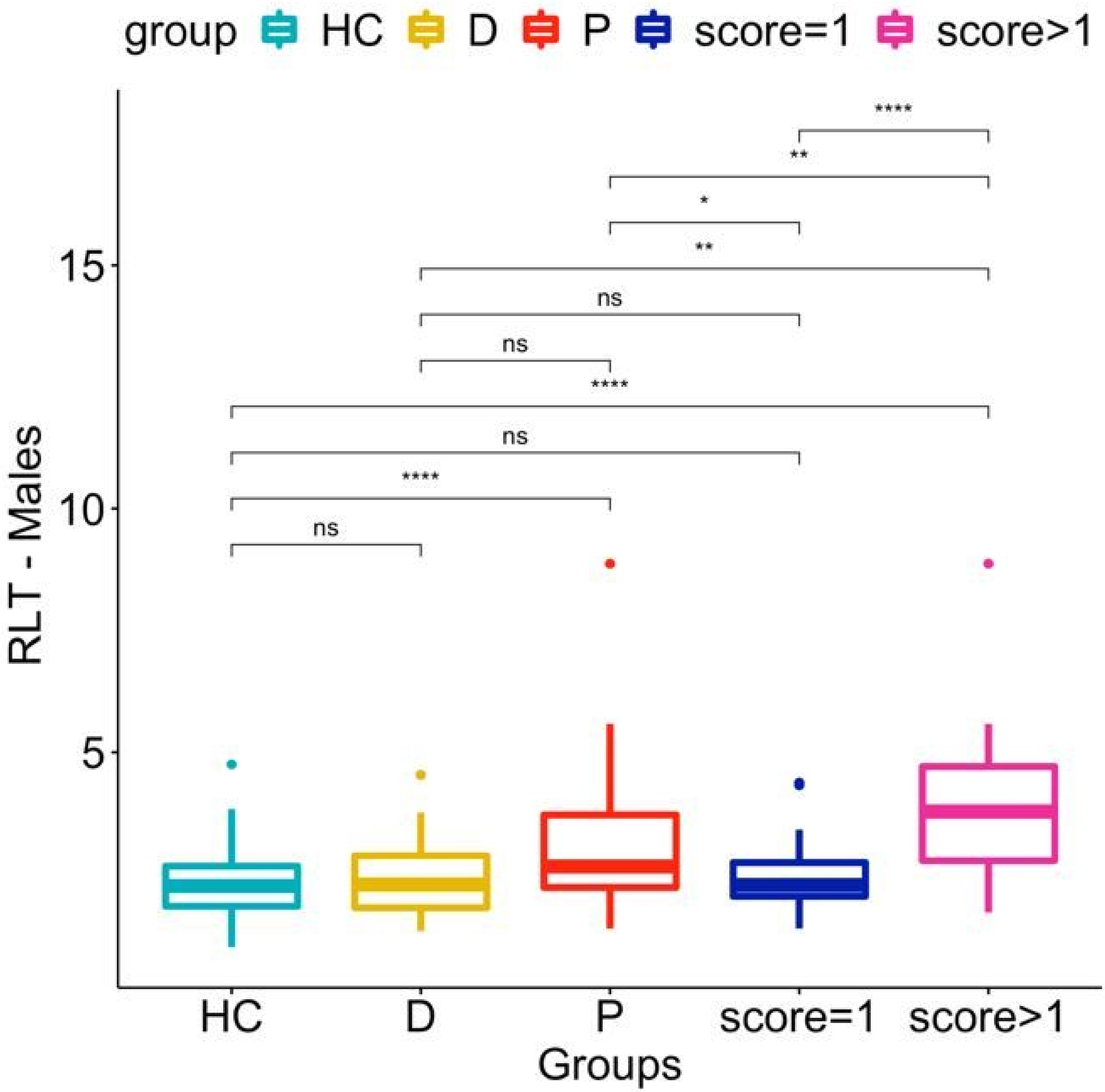

Figure 6

Distribution of RLT values for healthy controls (HC), doubtful cases (D), patients (P), patients with score = 1, and patients with scores >1 across the male population. ns: p > 0.05 ; * p ≤0.05; ** p ≤0.01; *** p ≤0.001; **** p ≤0.0001.

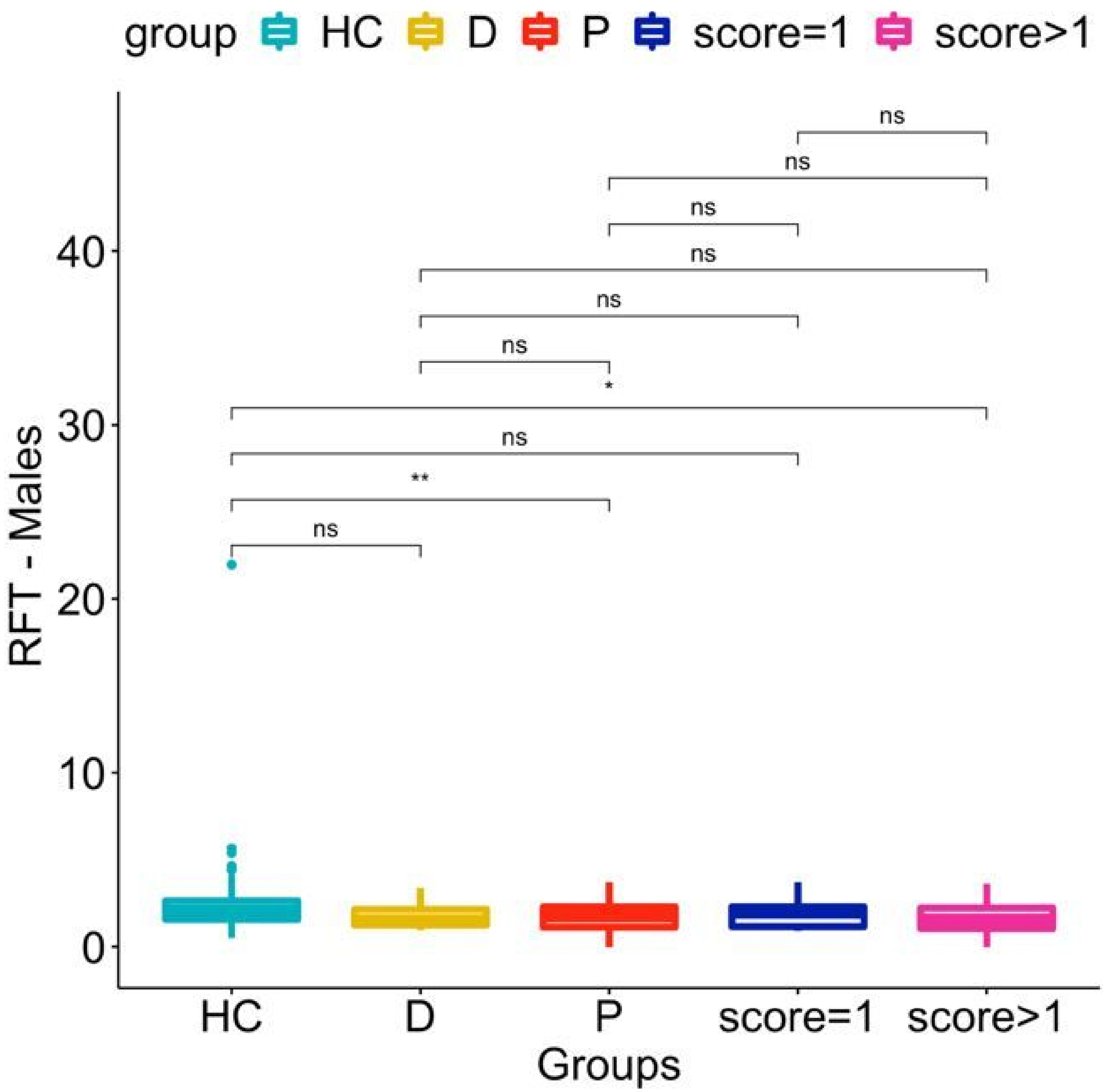

Figure 7

Distribution of RFT values for healthy controls (HC), doubtful cases (D), patients (P), patients with score = 1, and patients with scores >1 across the male population. ns: p > 0.05 ; * p ≤0.05; ** p ≤0.01; *** p ≤0.001; **** p ≤0.0001.