Microcalcifications and punctate echogenic foci, how well do we know what we are seeing?

Abstract: The presence of microcalcifications in thyroid nodules is a very specific sign of malignancy, as it corresponds to Psammoma bodies. There are not enough studies that demonstrate a correlation between their histological presence and their actual ultrasound appearance. Materials and Methods: All nodules larger than 3 cm punctured at the Universidad Católica Clinical Hospital between 2010-2015 were selected, and the sonographic appearance was classified according to the presence of 3 types of echogenic foci according to a stricter definition than usual. The above was correlated with findings in biopsies. Results: 44 nodules corresponded to papillary thyroid cancer. There was a statistically significant relationship between a new ultrasound definition of the microcalcifications (punctate echogenic foci) and the histological presence of psamomma bodies. Discussion: There would be a good correlation between a stricter definition and the actual presence of microcalcifications in histology, improving the high rate of over-diagnosis recently noticed by some authors. Keywords: Thyroid, Ultrasound, Microcalcifications, Psamomma bodies

Resumen. La presencia de microcalcificaciones en nódulos tiroideos es un signo muy específico de malignidad, al corresponder a cuerpos de Psammoma. No existen suficientes estudios que demuestren una correlación entre su presencia histológica y su aspecto ecográfico real. Materiales y métodos: Se seleccionaron todos los nódulos con tamaño mayor a 3 cm puncionados en el Hospital Clínico Universidad Católica entre los años 2010-2015 y se clasificó el aspecto ecográfico según la presencia de 3 tipos de focos ecogénicos según una definición más estricta a lo usual. Se correlacionó lo anterior con hallazgos en biopsias. Resultados: 44 nódulos correspondieron a cáncer papilar de tiroides. Hubo relación estadísticamente significativa entre una nueva definición ecográfica de las microcalcificaciones (focos ecogénicos puntiformes) y la presencia histológica de cuerpos de psamomma. Discusión: Habría una buena correlación entre una definición más estricta y la presencia real de microcalcificaciones en histología, mejorando la alta tasa de sobrediagnóstico advertido recientemente por algunos autores. Palabras clave: Tiroideas, Ultrasonido, Microcalcificaciones, Cuerpos de psamomma.

Introduction
On ultrasound, microcalcifications in thyroid nodules and their relevance for diagnosis have been widely studied, there being a strong association between their presence and the risk of malignancy, especially in relation to papillary thyroid carcinoma (PTC). From an anatomopathological point of view, they are considered to represent the presence of psamomma...
bodies (PBs) (Figure 1) and can be located in nodules as well as in the thyroid parenchyma. They correspond to papillary structures, which after a process of necrosis evolve into a dystrophic calcification with a laminated and concentric morphology. They are commonly observed in the PTC, being rarely reported in other neoplastic and non-neoplastic lesions. However, the echographic appearance of the microcalcifications can be confused with echogenic foci of another nature, there being a high interobserver variability in the diagnosis of them, a subject that has not been sufficiently studied, but empirically there is an impression of a marked tendency to over-diagnosis.

Among the multiple factors that can explain the problem, one of the most important is the absence of evidence that demonstrates a correlation between what is classically accepted as microcalcifications on ultrasound and the actual presence of PBs.

**Figure 1.** A fragmented microcalcification corresponding to a Psamomma body is observed at the center of the image.

**Objective**

To propose a classification of the echogenic foci visualized by ultrasound in thyroid nodules, which are generally considered microcalcifications, defining in a more precise way which are the ones that are most likely associated with PBs, making the echo/cyto-histological correlation of microcalcifications in nodules of patients with papillary thyroid cancer, in order to establish the diagnostic accuracy of the ultrasound method and the degree of statistical correlation between the microcalcifications, now with a stricter definition (echogenic foci type 1) and the actual presence of PBs in the histopathological examination.

**Materials and methods**

A retrospective study included within another larger study in relation to the ultrasonographic characteristics of thyroid nodules larger than 3 cm. We reviewed puncture ultrasounds with their reports, result of cytologies (FNAC) and biopsies of nodules >3cm between January 2010 and December 2015 available in the PACS-RIS® system of the UC-Christus health network.

44 patients with PTC were included for the statistical analysis. The selected population corresponded to patients with PTC in FNAC with subsequent confirmation with surgical biopsy or concordant clinical follow-up in patients not candidates for surgical resection.

With respect to the search for PBs, we reviewed in detail the pathological anatomy reports of the FNACs of the 44 nodules, and the slides of surgical biopsies carried out in our institution were re-analyzed (n= 16). The slide revision was carried out by two trained operators with 2-3 years of experience, and certified by an anatomopathologist with 15 years of experience in thyroid pathology.

The data collection in relation to the ultrasound reports was carried out by two operators with 1.5 and 3 years of experience on the subject, selected and trained by a radiologist with 37 years of experience in thyroid ultrasound, in the classification and characterization of thyroid nodules. We reviewed 4,462 ultrasound puncture scans between the dates described and selected all nodules with a size >3cm. The ultrasound characteristics and concordance with the reports were recorded, being reviewed by the experienced operator, all the cases with report-image mismatch or those in which no report was found and the trained operators considered that they required review by the expert for the final determination of the characteristics of the nodule. As a quality control in relation to the present work, a blind review by the expert was requested for the 44 cases with PTC of the echogenic foci under study.

**Classification of the different echogenic foci**

- Regarding the ultrasound classification used for the different types of foci, the last consensus for the ultrasonographic classification of thyroid nodules proposed by the American College of Radiology was used as the basis and an adaptation was made in order to resolve the conflicts that it poses, arriving at the following definitions:

  - **Type 1:** Punctate echogenic foci (PEF) equivalent to microcalcifications according to this consensus, defined as “punctate echogenic foci with a size smaller than 1 mm, without acoustic shadow”. To the above proposed by the ACR, we add some features that help with the diagnosis: when they are grouped preferably in the thickness of the solid tissue they are aligned in the periphery of the nodule and when they appear with a “salt and pepper” pattern. Exceptionally, if a conglomerate of microcalcifications is grouped focally it can generate an acoustic shadow and this does not
rule them out (Figures 2, 3, 4 and 5).

- Type 2: Indeterminate echogenic foci (IEF) defined as foci of a larger size (1-2mm), of greater echogenicity than PEF, without comet-tail artifact or <1mm rather isolated and in relation to microcysts (Figures 6 and 7).
- Type 3: Thick colloid foci defined by the presence of a “comet-tail” artifact greater than 1mm and the rest of the sonographic characteristics similar to the IEF (Figures 8 and 9).

Figure 2. Punctate echogenic foci (PEF), as proposed by our definition.

Figure 3. Echogenic foci type one, in the periphery of the nodule.

Figure 4. Punctate echogenic foci with a “salt and pepper” pattern.

Figure 5. Conglomerate of echogenic foci with acoustic shadow behind the group.

Figure 6. Indeterminate echogenic foci (IEF) are observed randomly distributed, with echogenicity greater than that of PEF.
**Results**

Within the general group of the study (n= 455) initially the total PTC was obtained, corresponding to 44 nodules (9.67%) with a size >3cm. The frequency distribution of the different echogenic foci was obtained according to the adopted definition [95% CI] for the general group, as can be seen in Table 1. The degrees of significance and statistical correlation between various combinations of echogenic foci and malignancy or PTC were calculated, as shown in Tables 2 and 3. The association between thick colloid and benign foci was also calculated to compare with the literature reviewed, obtaining a statistically insignificant difference with the control group (X² 1.775; p= 0.183) and an insufficient correlation coefficient (Cramer’s V) (0.062).

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<th>Table 1. Distribution of the different echogenic foci studied in the general group of nodules &gt;3 cm (n= 455).</th>
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<td><strong>Frequency</strong></td>
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<td>Without foci</td>
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<td>PEF</td>
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In the specific group of nodules with PTC, we obtained: 14 nodules without foci; 25 with type 1 foci (PEF); 5 with type 2 foci (IEF); none with thick colloidal foci. Regarding the percentage distribution of echogenic foci, there was a statistically significant difference (p <0.05) compared to the general group, observing a significant increase of PEF and a marked decrease of IEF and thick colloid foci in the PTC group, as seen in Figure 10. As relevant supplementary information related to the correct detection and differentiation of the different echogenic foci, there were no cystic nodules and only 2/44 were hyperechogenic.

Table 4. Diagnostic evaluation of our PEF definition for PBs (real microcalcifications).

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<td>Sensitivity</td>
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<td>Specificity</td>
<td>97%</td>
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<td>PPV</td>
<td>40%</td>
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<td>NPV</td>
<td>99%</td>
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Discussion

On ultrasound, the finding of microcalcifications in a thyroid nodule is a high suspicion sign for thyroid cancer, principally PTC\cite{1,2}, the most frequent variety of thyroid cancer\cite{12}, especially if they are associated with solid composition and hypoechogenicity. Their presence will almost invariably be a reason to indicate a FAP which is currently the best way to advance in the diagnosis. However, most of the echogenic foci do not correspond to microcalcifications and are very similar to each other, being necessary to specify which characteristics are those that most likely correspond to microcalcifications, with PBs in their histopathological correlation.

There is no consensus in literature regarding the nomenclature of the different echogenic foci, nor the precise pictographic definition for each of them\cite{6-11}. Recently, a study published by a group with vast experience in thyroid pathology\cite{5}, suggested for the first time a new approach to solve some of the questions surrounding the issue, evaluating the degree of association between five different types of proposed echogenic foci and the risk of malignancy\cite{5}. In that study, a high proportion of nodules with echogenic foci was found, which had no greater percentage of malignancy compared to the group without echogenic foci (nodules with echogenic foci; 14.9%; nodules without echogenic foci, 12.2%, p= 0.31). However, when separating the foci with comet-tail artifact greater than 1mm from the other types of foci, a very strong association with benignity was observed, unlike the remaining group that taken as a whole, showed a higher rate of neoplasms. Even so, what was defined in that study as “punctate echogenic foci” (equivalent to what most other works call “microcalcifications”) showed a discreet increase in risk, observing the presence of these elements in 89.4% of all the nodules with echogenic foci. Of the latter, 84.4% corresponded to benign nodules. Consequently, a serious error is considered in the nomenclature used to date, for using the term microcalcifications with such a degree of laxity, since there is no reason to think that you could find PBs (true microcalcifications).

With respect to the effective presence of PBs in histology, it was found that in total 14 thyroid nodules with PTC had such calcification present. Of the PBs found 9 were only detected in surgical biopsy; one detected in biopsy and cell block; 4 detected only in cell block, although in these last 4 cases it was not possible to access the surgical biopsy slides. Of these, 13 nodules (92.86%) were correctly classified with ultrasound (PEF); there was one false negative (7.14%); it was classified as without echogenic foci); there were no PBs in nodules with IEF.

To evaluate the diagnostic performance of the ultrasound to correctly detect PBs, the results of the cyto-histology were used as diagnostic Gold Standard, obtaining the sensitivity, specificity, PPV and NPV for our definitions with the results indicated in Table 4. It can be seen that sensitivity, specificity and NPV show positive results, however, a poor PPV was obtained.

Figure 10. Comparative percentage distribution between the presence of the different echogenic foci in the general group (black) vs the PTC group (gray).
in such a high percentage of nodules14, which are also usually colloidal15.

Hence the initiative to propose three types of echogenic foci to simplify the observation, being type 1 (PEF) the most characteristic of microcalcifications, type 3 characteristic of thick or crystallized colloid, and type 2 (IEF) as an indeterminate group without meeting criteria to define it as type 1 or 3, in which good sense recommends bring cautious in describing them as microcalcifications (Figura 11), since this significantly increases the score in the risk stratification systems currently used (TIRADS, ATA)6-11, generating a possibly unnecessary FAP. Thus, the objective is to improve the specificity in the diagnosis of microcalcifications using stricter criteria. In daily practice it is common to see in the ultrasound reports the echogenic foci being indiscriminately assumed as microcalcifications, in accordance with what was found in the work mentioned above. The degree of over-diagnosis can be such, that certain authors have come to propose only using the term “echogenic foci” in a general way without differentiating between them13.

In order to support this approach, we initially obtained the results shown in Tables 2 and 3, which showed a moderate-high correlation between PEF/ malignancy and PEF/PTC, indicating a better performance compared to the results of other studies. It can be seen in the same table that there was no significant correlation between the other echogenic foci and malignancy or PTC, favoring the approach that the fact of correctly identifying microcalcifications on ultrasound does have an implication in the management of these patients. This last point gains much more force when observing what happens on mixing PEF and IEF as if they were a single entity (PEF+IEF in Tables 2 and 3), seeing that a significant difference persists with the control group regarding the risk of malignancy or PTC, however, the degree of statistical correlation that could be considered significant is totally lost (Cramer’s V <0.3). The colloid value was also evaluated as a predictor of benignity, however, there was no statistically significant correlation (p>0.05). This last point could be explained by the small size of this group (5.71% of total nodules), although it was a proportion equivalent to that reported in other series.

**Performance of the proposed definitions and presence of Psamomma bodies**

Comparatively between the general group and only the PTCs, there was a marked difference in the distribution of PEF vs IEF (Figure 6), supporting that the proposed definition with the aim of improving the detection of PBs could be useful, considering that these theoretically should be presented almost exclusively in nodules with PTC6.

In our study, we found that of the 44 cytologies, 5 (11.36%) detected the presence of PBs; of the 16 surgical biopsies available for slide review, 10 (62.5%) showed PBs. Although in this context no comparison can be made between the two study techniques, there is a tendency to a higher probability of detecting PBs in the biopsy vs cytology, which is consistent with results of previous studies. It is worth mentioning that 4 biopsies of nodules were analyzed whose ultrasonography reported absence of echogenic foci, which confirmed the absence of PBs (this data was not included in the results due to its small number). When doing the inverse correlation between PBs in biopsy and ultrasound appearance, there was only one false negative, confirming that the ultrasound is exposed to false negatives and that the detection of microcalcifications depends on the number and location, as well as the composition of the nodule.

With respect to what was defined by our group as IEF, a good capacity of this term to differentiate the echogenic foci that do not correspond to microcalcifications is observed, with no PBs existing in the histology for any of that group. However, we do
not know exactly what they would correspond to. Among the possibilities, it is found that they correspond to posterior walls of microcysts, colloid foci whose amount is not enough to generate comet-tail artifact >1mm, fibrotic foci or others. Even so, in the context of the results obtained, this differentiation does not seem to be relevant since no significant association with malignancy or PTC is observed in this study. Given that in our definitions we did not include the comet-tail artifact <1mm, and consequently, those were included within the IEF, it could be that the correlation indicator between IEF and malignancy or PTC is overestimated despite its non-significant value in our statistical analysis and, in turn, that we have not been able to isolate the possible value of these types of foci described in the literature as having a statistically significant difference with the control group in terms of the risk of malignancy. The latter does not seem to be an important limitation of the study since it was not within the initially proposed objectives, and it is also described that its presence, although it would be significant in terms of the risk of malignancy, is in a low proportion of thyroid nodules.

To evaluate the clinical and diagnostic value of our strict definition of PEF in relation to PBs, sensitivity, specificity and predictive values were calculated, with the results indicated in Table 4. As can be seen, our definition shows a very good performance for differentiating those nodules that actually have PBs versus those that do not. One clear aspect to improve upon is the PPV, given that despite having made much more stringent the criteria for reporting PEF, a marked over-diagnosis was maintained (13 true positives vs 12 false positives), although lower than in any other study available at present.

The cyto-histology was used as a diagnostic gold standard, considering the ample evidence in relation to the capacity of cytology and biopsy for the final diagnosis in thyroid nodules.

Unfortunately, surgical biopsies of all patients with PTC could not be accessed for multiple reasons, observing in the 16 analyzed a detection rate much higher than the detection rate of PBs from mere cytology. Considering this last point, it can be concluded that there is an important possibility that the proposed definition has a PPV much better than that obtained.

We mentioned in the results the distribution regarding the echogenicity and composition of the studied nodules, given that these factors could have contributed to a better detection of PEF compared with other series in which there is a greater proportion of nodules with a cystic component >50%, as well as a greater number of hyperechogenic nodules. In our series, in the group of 44 PTC, we obtained: 2 hyperechoic nodules (4.5%); 12 isoechoic (27.3%); 30 hypoechoic (68.1%); 16 predominantly solid nodules (36.4%); 28 solid nodules (63.6%). We do not know if this distribution is comparable or not with the PTC of different sizes.

Our study presents multiple and important limitations in the context of a retrospective design. These include the absence of standardization regarding the type of ultrasound beam used (multidirectional vs. linear), which can determine an important difference in the detection of acoustic shadow and microcyst differentiation, which in turn could be the cause of generation of the hyperechogenic foci, in addition to the use of equipment from two different manufacturers. The results were obtained only from the group of nodules larger than 3 cm, which can indicate an important selection bias. Likewise, the determination of malignancy/benignity was mostly based on cytology results without confirmation with surgical biopsy, although FAP is considered as a very high sensitivity and specificity test for Bethesda V and VI categories. Regarding our definitions, despite the good results obtained, these have a subjective component that is not insignificant (problem inherent in the examination given its operator-dependent nature), which indicates that the best performance compared to other series may be due significantly to the fact that the final determination of the echogenic foci was based on the diagnosis made by an expert operator, being possible that our results are not reproducible by radiologists with less experience. Finally, the biggest limitation of our study was the availability of only 16 surgical biopsy slides for review, there being a very high risk of bias since it was not possible to include 28 biopsies. We believe that, although cytology helped to give 4 additional cases with PBs to those provided by the analyzed biopsies, the real gold standard for the detection of PBs may be surgical biopsy, so the total number of patients with PBs could be very underestimated.

Conclusions

Despite the limitations of our study, we observed a possibility to improve the problem of over-diagnosis of real microcalcifications (PBs) by reformulating the definition of microcalcifications with stricter criteria. In our series there is a good correlation between PEF and PBs, although the resulting PPV indicates the need to further define its appearance.

To date, there is no certainty as to the actual clinical relevance of making a strict differentiation of the multiple echogenic foci found in nodules (except those with comet-tail artifact >1mm as suggested in several other series). However, we believe that this is due to the lack of an accurate pictographic
definition of these different elements and that isolating the appearance of microcalcifications is relevant, as our results and some histological studies indicated. Thus, there is a clear need to design and implement prospective studies that solidly prove our hypotheses, with the intention of reaching a modification of the current paradigm.

References
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