



Insidious Rare Thyroid Microcarcinoma (Black Ink)

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ABSTRACT Thyroid cancer is among the most common cancer in women and new cases continue to rise in all the world. The incidence of this cancer is kept under control mostly by surgery. There are more rare subtypes of thyroid cancer as papillary microcarcinoma (black ink) that even small are aggressive and there is the need to early diagnosis to avoid their insidious behaviour. Thirteen patients have been selected and studied

KEYWORDS : Thyroid, Ultrasonography, Elastasonography, Microcarcinoma, Black Ink

Introduction

The thyroid carcinoma is the most frequent endocrine cancer and represents 1% of all malignant lesions. Its incidence in the general population varies from 0.05 to 1 new case per 100.000 in habitants every years. By definition of the WHO (World Health Organization)¹ the papillary microcarcinoma is a malignant thyroid cancer with a diameter that does not exceed 1 cm. As for all the thyroid cancer the cause of papillary carcinoma is unknown.

However, there are risk factors for the development of the thyroid cancers (ionizing radiation, iodine deficiency, autoimmunity , familiarity)².

The mutation of the BRAF³ gene, and the RET / PTC⁴ rearrangement represent the genetic alterations that are more frequently associated with the thyroid papillary carcinoma.

Currently this new neoplasm identified as micro-focus, it has been proved rare and insidious, it is called "black ink", classified as papillary microcarcinoma (Fig 1, 2).

It hits all the ages and prefers the female sex. Thyroid microcarcinoma can be diagnosed occasionally, or it is a common finding with autopsy. Several lines of evidence suggest the possibility that the thyroid carcinomas of small dimensions may show strong biological aggressiveness⁵.

The ultrasonography represents the more sensitive diagnostic technique of images for an early diagnosis of the thyroid lesion.

The echographic aspects do not have adequate sensibility and specificity for a malignancy diagnosis. The predictive value of these criteria is not enough high or low to rule out the need of the FNAC.

The FNAC (Fine Needle Aspiration Cytology) allows the diagnosis of the thyroid lesion type with a diagnostic accuracy of 92-95%⁶. It has two important limitations: inadequacy of the samples for cytology and follicular proliferation.

These limits could be substituted by the introduction of new methods able to differentiate with high accuracy the malignant and benign lesions by the use of ADF (Advanced Dynamic Flow) and elastasonography. The ADF allows to underline the vessel flow of the newly formed tortuous vessels (Fig. 3).

Elastasonography allows to analyse the mechanical-elastic property of the soft tissue through the deformation or distortion of the tissue in response to a compression applied externally, obtained thanks to a software (Toshiba Elasto US)

an algorithm that measures the degree of elasticity or hardness of the

tested lesion.

In the post-operative follow-up one patient with the microlesion presented a recurrence in the lymph nodes.

A careful histological examination had showed multifocal in one patient, capsular infiltration in anotherone, and a gelatinous-like structure in all the 13 patients.

This study has identified major risk factors: dimension of the lesion, infiltration of the capsule, multifocality, recurrence in the lymph nodes.

Aim

The purpose of the study performed on 13 microcarcinoma, called black ink, is to identify the sonographic features, ADF, elastasonography, cytological and histological observations as "prognostic factors" to define the optimal therapeutic strategy.

Material and Methods

From January 2011 to March 2013 380 caucasian patients were enrolled, 273 females and 107 males, (age between: 33/71) for the ultrasonographic study of thyroid echostructure abnormality. Each patient has been subjected to bidimensional ultrasonography (DUS 2) using high frequency probes (13 -15 MHz) (Toshiba Aplio 500) (Ariet et al. -2016).

The parameters of detected lesions were studied, carefully as follows: topographic location, level of echogenicity, shape, echostructure, definition of margins, size, presence of microcalcification, assessment of vascularization, elastasonographic study and FNAC ecoguided by free hand.

In 13 of 380 patients, 11 females and 2 males, a unilateral solitary micro-focus has been identified, of radial shape, with markedly hypoechoic echostructure, showing irregular and infiltrative margins, without microcalcifications, with black ink aspect (range: 4-7mm).

Subsequently it has been carried out a diagnostic work with new technologies:

ADF test, elastasonography and cytological biopsy with needle at a variable diameter (22/24 G - ECOJEKT DIVA TM - HS Hospital Service - Italy).

ADF –Advanced Dynamic Flow

The presence and pattern of blood flow assessed by ADF (Advanced Dynamic Flow), "the study of the advanced dynamic flow" allows to measure accurately the vascular system.

It was classified as follows:

No ADF signal in the surroundings or in the micro-focus;

peripheral vascularity defined as flow in a peripheral position and absent or slight flow in the central part of the micro-focus; marked intralobular vascularity defined as more flow in the central part of the micro-focus compared to the periphery; mixed vascularity defined as equivalent flow both in the peripheral and central micro-focus.

Elastosonography

Elastosonography was performed after conventional ultrasonographic examination (Fig 4).

The probe was placed on the neck with a light pressure, and an elastographic region of interest (ROI) has been placed on a micro-focus with a sufficient surrounding thyroid tissue to evaluate (Fig 5).

Keep uniform the distribution of the effort the probe has been pressed on the area with a frequency of 3 to 4 times a second during the compression-decompression cycle of the elastosonography.

The elastogram in real-time has been displayed as an overlay on the gray scale imaging and a color-coded map.

Highly elastic tissues (soft) appear in red, less elastic tissues appear in blue (hard), the intermediate degrees of elastic tissues are shown in green.

The elastosonography images were classified according to Hong score⁷, in a score of 1- 5, (Tsukuba Elasticity Score Patterns - Table A). In this study, malignant lesions as demonstrated by Hong, with a score of 3 - 5, were considered as "hard" malignancy, and the remaining score as soft (Table B).

Biopsy and Cytology

The informed consent has been obtained from all patients before their exam.

In all 13 patients an examination has been performed with a guided FNAC exam, leaving the task to the cytology for characterizing the lesion (Fig 6).

In patients with preoperative diagnosis of malignancy, the elective surgery has been a total extracapsular thyroidectomy, the central node dissection, with the unilateral lymphadenectomy (modified lymphadenectomy of the neck) following the technique described by Lahey⁸ (Fig 7, 8).

The histological diagnosis was performed in accordance to the criteria established by the WHO¹ and the Armed Forces Institute of Pathology⁹ (Table C and D). After one month from the total thyroidectomy it has been performed a whole body scan.

The patients were subjected to hormonal and cancer markers control (thyroglobulin, anti-Tg antibodies), ultrasound evaluation of the neck after 3 months.

The period of follow up has varied from a maximum of 2 years to a minimum of 1 year.

Results and Discussion

The DUS 2 ultrasound with high resolution is considered the first line method for the study of ultrasound characteristics of this new lesion, called "Black Ink".

Size, shape, level of echogenicity, margins, presence of microcalcification, topographical localization.

The sonographic aspect of malignant characteristics of thyroid papillary microcarcinoma (PTMC), have been defined as those showing at least one accepted ultrasound criterion described above¹⁰.

The identified micro-focus was unilateral, localized in the middle third of the right or left lobe, presenting a radial shape, and markedly hypoechoic echostructure, with irregular and infiltrative margins.

The ADF has identified accurately the microvasculature of the micro-focus.

The vessels within the cancer are small with a slow flow and the flow signal, detectable by ADF is only detected at the periphery.

Elastography, a non-invasive technique, was introduced to evaluate the tissue texture and it is useful in differentiating benign and malignant thyroid lesions^{7,11,12}.

It has been used the real-time method of elastosonography (free hand) and software Toshiba (Strain Elastic sonography) during the ultrasonography examination.

The deformity (strain) of thyroid micro-focus has been evaluated and the strain index (ratio of strain between normal and thyroid cancer).

A value of strain index greater than 4-5, it is significantly associated with malignancy¹³.

The micro-focus designed by the elastography showed a very high degree of score, Score: 5.89 with a high predictive value for malignancy ($p < 0.001$), with a sensitivity of 89%, a specificity of 79%, a positive predictive value of 73% and a negative predictive value of 92%.

All the micro-focus analysed at FNAC were TIR 5 according to the Italian classification of Pathological Anatomy and Diagnostic Cytopathology (SIAPEC 2014) in accordance with the Bethesda Classification (Table C and D).

The histological findings of micro-focus were all classified as papillary carcinomas, PT1 and PT3. (Table E and F).

The interest for this particular cancer of the thyroid is due to its rarity and especially for an aggressive biological behaviour.

Several previous studies have shown the various factors that influence the aggressive biological behavior of thyroid microcarcinoma^{14,15}.

There are three possible diagnosis of a thyroid microcarcinoma: Incidental finding in thyroids removed for benign disease, histologic diagnosis of metastatic thyroid cancer in the neck lymph nodes, cytological diagnosis by FNAC^{16,17,18}.

Woolner in 1959 considered the PTMC a low-grade lesion of malignancy (as demonstrated by autopsy finding)¹⁹ and argued that the life expectancy of patients suffering from this cancer did not differ from that of the general population.

Other Authors^{20, 21} argue that the difference of the papillary microcarcinoma is only to the size in comparison with other thyroid cancer, and not for morphological, clinical and prognostic characteristics, they report cases of recurrence and aggressive behaviour with multiple metastases²².

The conclusion of these different opinions is that the microcarcinoma may not be always a lesion of "low risk", but that there are also cases with more severe prognosis.

These identified micro-focus, have different biological behaviour compared to the PTMC not detected early, and will express a higher index of the disease aggressiveness and will be predictive of possible local and distant recurrence.

According to Hay two are the important parameters to keep in mind in the possible onset of a recurrence: multifocality (number of focus) and the type of surgical treatment (total thyroidectomy versus lobectomy)^{23,24}.

Kasai and Saramoto²⁵, claim that the sizes of PTMC are makers of lymph node metastasis and vascular invasion.

Yokozoa and Ahuia²⁶ have respectively documented that 15.9% of cancers inferior to 1 centimetre show an extra thyroid invasiveness and that occult metastasis of thyroid cancer to the lymph nodes is up to 20% of cases.

By prognostic factors taken into account (Tab. E), one patient with "black ink" has shown lesions in the lymph nodes, multifocality was present in a second patient, the capsular infiltration in anotherone, the

presence of micro-focus to a gelatinous structure-like in all 13.

Conclusions

Our analysis of 13 patients where these micro-focus were identified, shows 4 important prognostic risk factors:

the dimension of the lesion, capsule infiltration, multifocal and recurrences in the lymph nodes.

Based on these results we can consider these histopathological criteria, prognostic factors of "high biologic risk" regarding the characteristics of this micro-focus "Black Ink".

Based on our experience we believe that the data from this study reveal that the micro-focus we have identified may be a new clinic entity in its own right, rare and insidious, because show important biological risk factors.

In fact, patients with micro-focus of 4 - 6 mm didn't show any recurrence during the follow up.

Micro-focus superior to 7 mm has been detected as relapse in the lymph nodes.

According to our study, the dimension of a micro-focus identified at an early stage is the important key to avoid serious biological consequences for the patient.

Therefore an early diagnosis of this new thyroid malignancy, would be an important tool for predicting the biological behaviour and would represent an extraordinary highly rated opportunity of good health for patients.

The authors declare no conflict of interests.

Acknowledgments:

The authors thank for their support: Foundation T & L de Beaumont Bonelli for Cancer Research Naples – Italy & University Hospital OO.RR. San Giovanni di Dio Ruggi d'Aragona – Medical School Salerno – Salerno - Italy.

Figure 1 Hypocogenic micro-focus, irregular and infiltrative margins

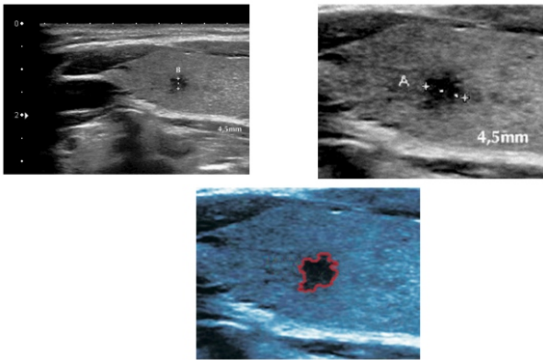


Figure 2 Micro-focus "Black Ink" Papillary Microcarcinoma (4,5 mm)

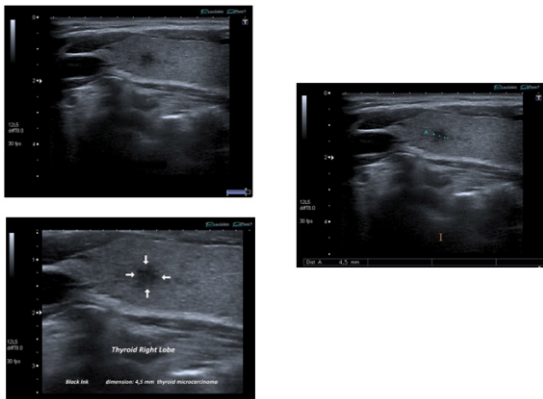


Figure 3 EcocolorDoppler Advanced Dynamic Flow -ADF

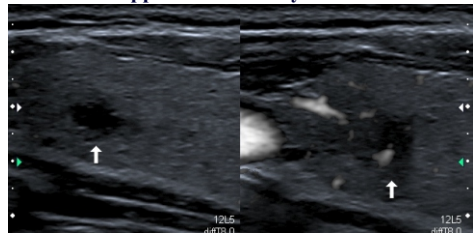


Figure 4 Elastasonography

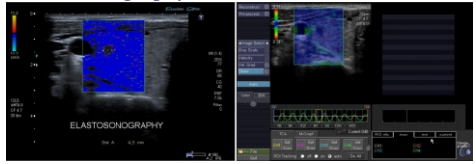


Figure 5 Elastasonography Curve Strain - (ROI)

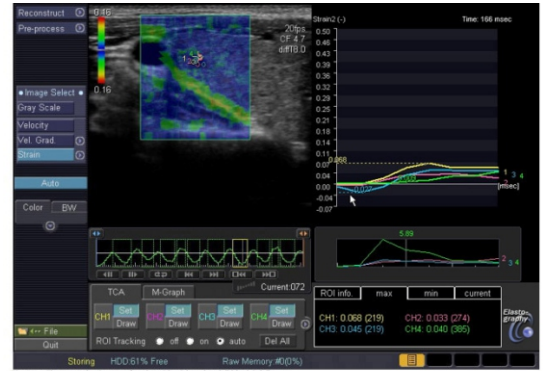


Figure 5. Elastography image of a thyroid with a "hard" "black ink" in blue color centrally located in the right lobe. The colored bar at the side of the image illustrates the degrees of relative stiffness harder tissues are shown in blue, and softer tissues range from green to red in color. A wave of sinusoids that shows the mechanical compression and decompression exerted in the study area is presented below the color image.

Figure 6 Biopsy and Cytology

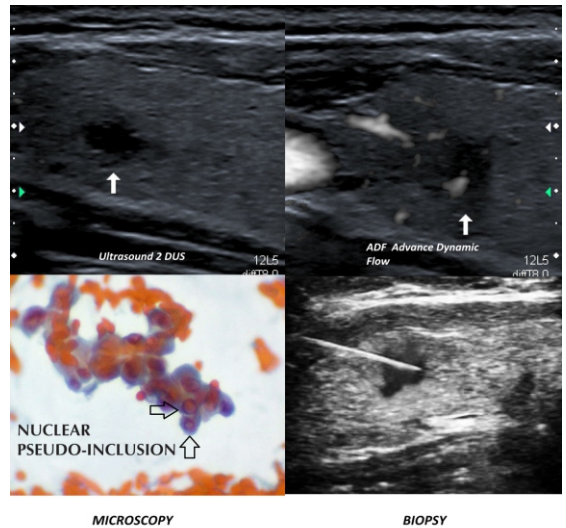


Figure 7 Macroscopic Pathology

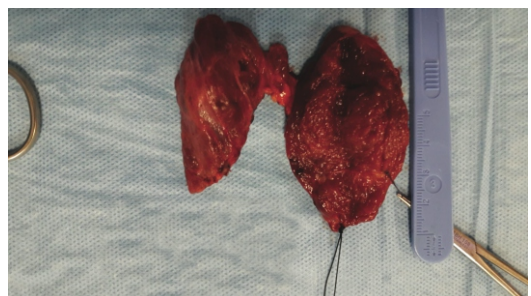


Figure 8

Table A Tsukuba Elasticity Score Patterns (scoring system for imaging interpretation)

Tsukuba Elasticity Score Patterns
Itoh A, Ueno E, Tohno E et al. Breast Disease: Clinical Application of US Elastography for Diagnosis. Radiology 2006; 239:341 - 350

Score	Classification Standard	Typical Image
1	Strain is seen in the entire hypoechoic area (the entire lesion is shown in green similar to the surrounding tissue)	
1*	BGR (blue-green-red) 3 layer pattern – typical artefact seen in a cystic lesion	
2	Strain is seen within most of the hypoechoic area but some areas show no strain (the lesion is a mixture of green and blue)	
3	Strain appears only in the periphery with no strain in the centre of the lesion (the centre of the lesion is shown as blue with the periphery in green)	
4	No strain is measured within the lesion (the entire lesion is shown in blue)	
5	No strain is measured within the lesion nor in the surrounding tissues (the lesion and the surrounding tissues are blue)	

Table B Elastosonography evaluation of thyroid with “Black Ink” and statistical data

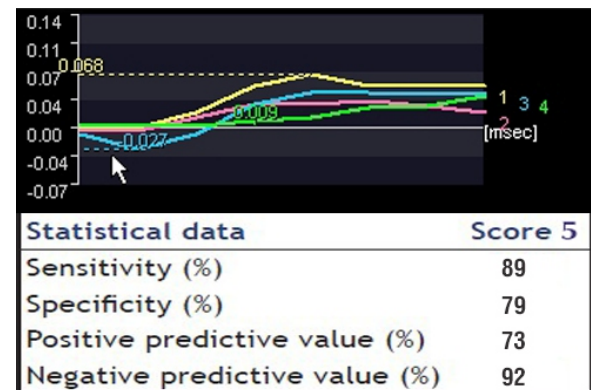


Table C Italian Classification of Pathological Anatomy and Diagnostic Cytopathology (SIAPEC 2014)

CODE	DIAGNOSIS CATEGORY	MALIGNANCY INDEX EXPECTED	TO DO
TIR1	No diagnosis possible	Not defined	Repeat needle biopsy ecoguided after at least one month
TIR1C	No diagnosis possible	Low (based on the clinical situation)	Re-consider the clinical situation and/or repeat the needle biopsy
TIR2	Not malicious / benign	< 3	Follow-up
TIR3A	Wound risk index low (LRIL)	< 10*	Repeat needle biopsy clinical follow-up
TIR3B	Wound risk index high (HRIIL)	15-30*	Surgery
TIR4	Suspected malignancy	60-80	Surgery (to consider, the analysis of frozen session)
TIR5	Malignancy	> 95	Surgery

* The malignancy expected rate for the undercategories TIR3 is principally originated by the clinical situation and is partially based on evidences of literature data.

Table D The Bethesda Classification System

Bethesda diagnostic category	British Thyroid Association	American Thyroid Association
I Non-diagnostic or unsatisfactory	Thy1 Non-diagnostic	Non-diagnostic/unsatisfactory
II Benign	Thy2 Non-neoplastic	Benign
III Atypia of undetermined significance or follicular lesion of undetermined significance	Thy3a Atypical features present	Indeterminate or suspicious for malignancy
IV Follicular neoplasm or suspicious for a follicular neoplasm	Thy3f Follicular neoplasm suspected	Indeterminate or suspicious for malignancy
V Suspicious for malignancy	Thy4 Suspicious of malignancy	Indeterminate or suspicious for malignancy
VI Malignant	Thy5 Diagnostic of malignancy	Malignant

The Bethesda System for reporting thyroid cytopathology: recommended diagnostic categories, implied risk of malignancy, and recommended clinical management. Risk percentages depend on rates of atypia and follicular lesion as reported by local pathologists

Table E Histology Table - Patients with Black Ink and recurrences (age: min/max)

Table H1

Pz	Sex	Age	Dimension	Surgery	Multif.	Infiltration Capsule	Micro-foc.	Limf. Rec.	TNM
S.A.	M	49	4,5 mm(dx)	TT+CL	NO	NO	YES	NO	PT1
M.S.	F	53	5,0 mm(dx)	TT+CL	NO	NO	YES	NO	PT1
A.M.	M	58	5,8 mm(dx)	TT+CL	NO	NO	YES	NO	PT1
S.S.	F	33	6,3 mm(sx)	TT+CL	NO	NO	YES	NO	T1
A.D.	F	40	6,5 mm(dx)	TT+CL	NO	NO	YES	NO	PT1
E.L.	F	63	7,1 mm(dx-an)	TT+CL+FUL	YES	YES	YES	SI 12 months	PT3m
I.A.	F	71	6,0 mm (sx)	TT+CL	NO	NO	YES	NO	PT3

TT: Total Thyroidectomy
CL: Central Lymphadenectomy
FUL: Functional Unilateral Lymphadenectomy

Table F Thyroid Cancer Survival

Table H2

Type of Thyroid Cancer	Survival in five years					Survival in ten years
	Stage I	Stage II	Stage III	Stage IV	Overall	Overall
Papillary	100%	100%	93%	51%	96%-97%	93%
Follicular	100%<	100%<	71%	50%	91%	85%
Medullary	100%<	98%	81%	28%	80%, 83% o 86%	75%
Anaplastic (often stage IV)			7%		7%-14%	(no data available)

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