





BRIEF REPORT

Cytological and molecular characterization of secretory breast carcinoma

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Abstract

Secretory breast carcinoma is a rare neoplasm, histologically well-characterized, and secondary to *ETV6-NTRK3* gene fusion, whose cytological features are scarcely described in the literature. We report the case of a woman with a history of secretory breast carcinoma 8 years before, who presented a periareolar nodule. A recurrence was diagnosed by fine-needle aspiration based on the cytomorphological features and pan-TRK immunocytochemistry on the cell block, and the patient underwent a mastectomy. The histology and molecular studies performed on the surgical specimen (immunohistochemistry, FISH and NGS) confirmed the diagnosis. Cytological smears showed abundant epithelial cellularity, in groups and single cells. These cells showed moderate atypia, with abundant cytoplasm. We observed intracytoplasmic inclusions and extracellular metachromatic globules. Immunocytochemical and immunohistochemical studies showed a triple negative breast tumour. NTRK overexpression was demonstrated with immunocytochemistry against pan-TRK on the cell block, as well as with immunohistochemistry in the surgical specimen. NTRK3 rearrangement was proved by FISH. In the primary tumour and in the recurrence, we demonstrated *ETV6-NTRK3* fusion by NGS. After conducting a literature review, we have found 26 articles describing the cytological features of secretory breast carcinoma in 33 patients. The smears were described as groups of epithelial cells with vacuolated cytoplasm, single signet ring cells and a globular extracellular secretion. In only two cases molecular confirmation of the diagnosis with *ETV6-NTRK3* fusion was proven, although not in the cytological specimen, but in the subsequent biopsy. The distinct cytological features of secretory breast carcinoma can help in its diagnosis, thus guiding the molecular studies. This is the first reported case that proves TRK overexpression, as a fusion surrogate, in the cytological sample.

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KEYWORDS

molecular diagnosis, NGS, NTRK, secretory breast carcinoma

1 | INTRODUCTION

Secretory breast carcinoma (SBC) is a very rare but distinct breast neoplasm that accounts for <0.05% of all breast cancers.¹ It predominantly affects women, with a mean patient age of 53 years,¹ although it was first described in children.²

Histologically, it is composed of epithelial cells with intracytoplasmic vacuoles and an extracellular eosinophilic bubbly secretion, both of which are PAS positive and diastase-resistant.³ Regarding immunohistochemistry, secretory carcinoma usually exhibits a triple negative profile, without expression of oestrogen receptor, progesterone receptor nor human epidermal growth factor receptor 2 (HER2). It normally expresses GATA3, SOX10 and S100.⁴ In spite of portraying a triple negative profile, SBC is associated with a favourable prognosis.⁵

SBC is well characterized molecularly by the pathognomonic *ETV6-NTRK3* gene fusion,⁶ with oncogenic activity and susceptible of treatment with TRK inhibitors.⁷ This fusion can be detected by fluorescent in situ hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR) and next generation sequencing (NGS) techniques.¹ Additionally, it is possible to suggest the presence of this fusion using immunohistochemistry with a pan-TRK antibody. With this technique, *NTRK3*-fused cases characteristically tend to show nuclear staining.³

Despite these distinct histomorphological and molecular features, SBC cytological features are scarcely described in the literature.

2 | CASE REPORT

We report the case of a 73 year-old woman with a history of a pT1cN0M0 secretory breast carcinoma (diagnosis based only on morphological criteria), treated with a lumpectomy with sentinel lymph node biopsy, and adjuvant radiotherapy (40 Gy) in 2012. Eight years later, she presented with a periareolar nodule.

On physical examination, a 1.5 cm firm palpable subcutaneous nodule, next to the surgical scar, was evident. A recurrence was diagnosed by fine needle aspiration. Cytological smears of the periareolar nodule showed abundant epithelial cellularity, arranged in groups and single cells. They showed moderate nuclear atypia, with prominent nucleoli and abundant dense polygonal cytoplasm, resembling a squamous neoplasm. We observed dense and metachromatic material, located as intracytoplasmic drops and as extracellular globules (Figure 1). The patient was treated with a mastectomy and axillary lymph node dissection. Given the favourable histology, she did not receive subsequent adjuvant chemotherapy.

A year later the patient developed elevated skin lesions, occupying a large area of the right chest wall, which on histologic

examination demonstrated dermic and lymphatic infiltration by secretory carcinoma. At this time, the patient was included in an open-label, single arm, phase 2 clinical trial (NCT04408118) with

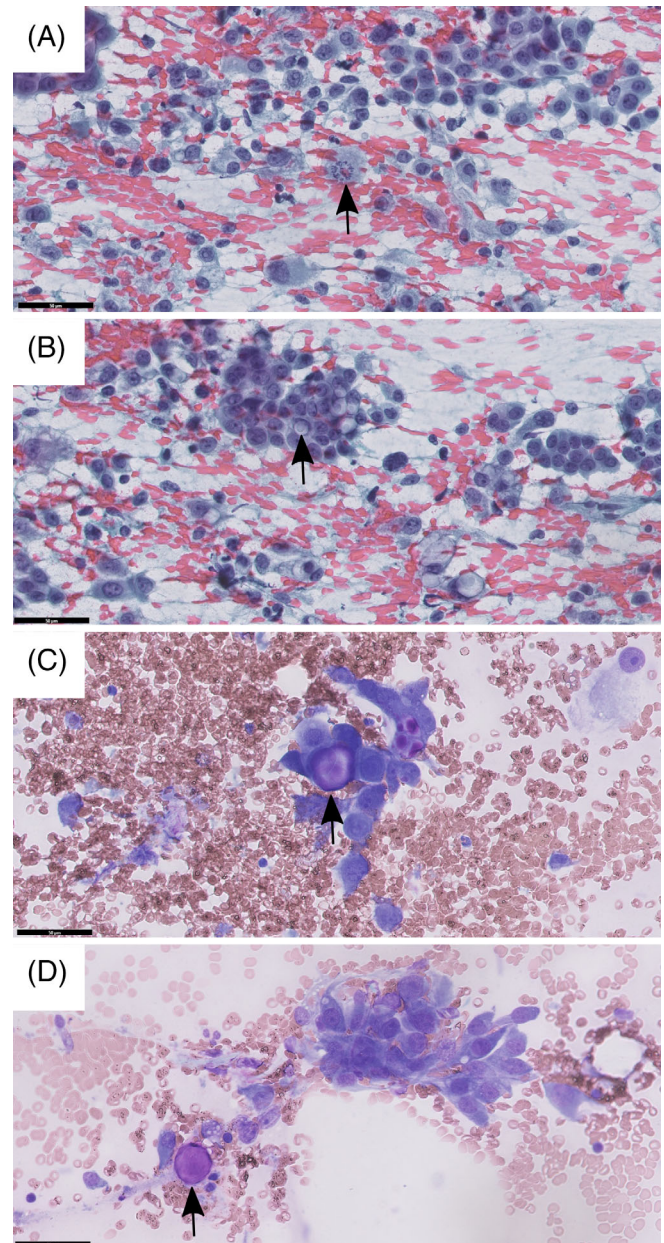


FIGURE 1 (A) May-Grünwald Giemsa. Cluster of atypical epithelioid cells with moderate nuclear atypia, prominent nucleoli and abundant squamous-like cytoplasm. A mitotic figure is shown (arrow). (B) Papanicolaou. Atypical cells with signet-ring features and intracytoplasmic drops (arrows). (C) May-Grünwald Giemsa. Atypical cells, one of them with signet ring features and an intracytoplasmic metachromatic drop (arrow). (D) May-Grünwald Giemsa. Atypical epithelioid cells next to an extracellular metachromatic secretion

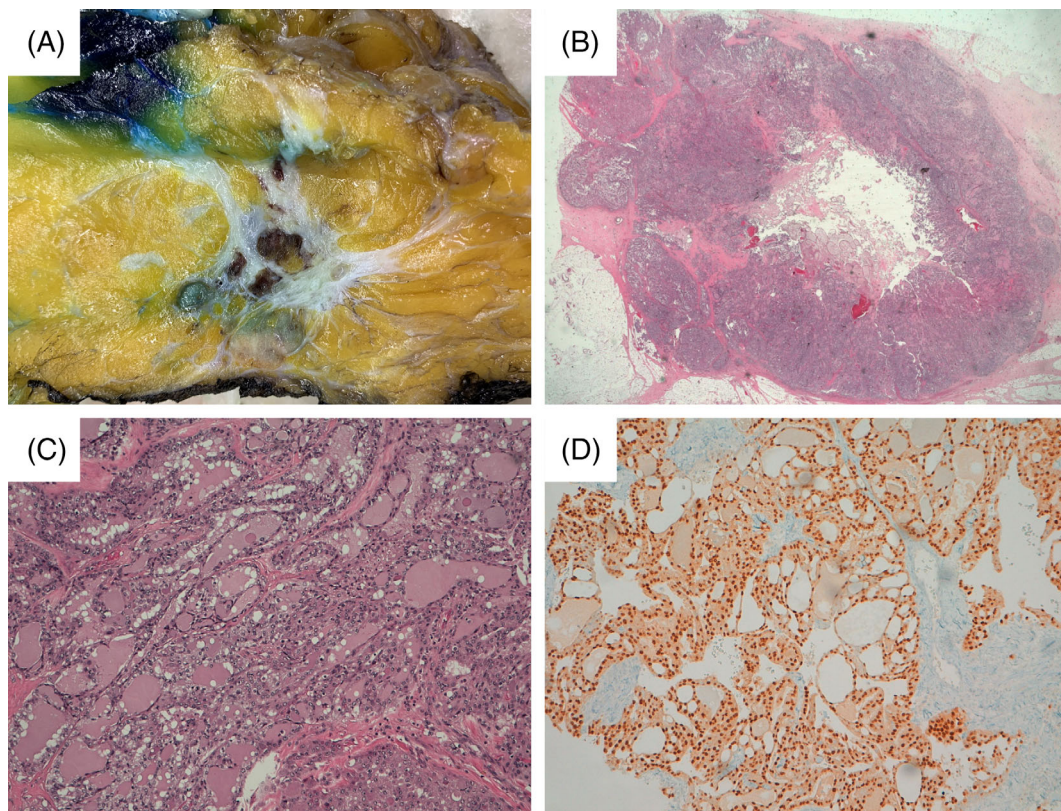


FIGURE 2 (A) Mastectomy specimen, showing a 2.2 cm ill-defined mass. (B) Panoramic view of the tumour. (C) Epithelial cells arranged in a microcytic pattern with eosinophilic secretions in a sclerotic stroma. (D) Pan-TRK immunohistochemistry showing strong nuclear and weak cytoplasm staining

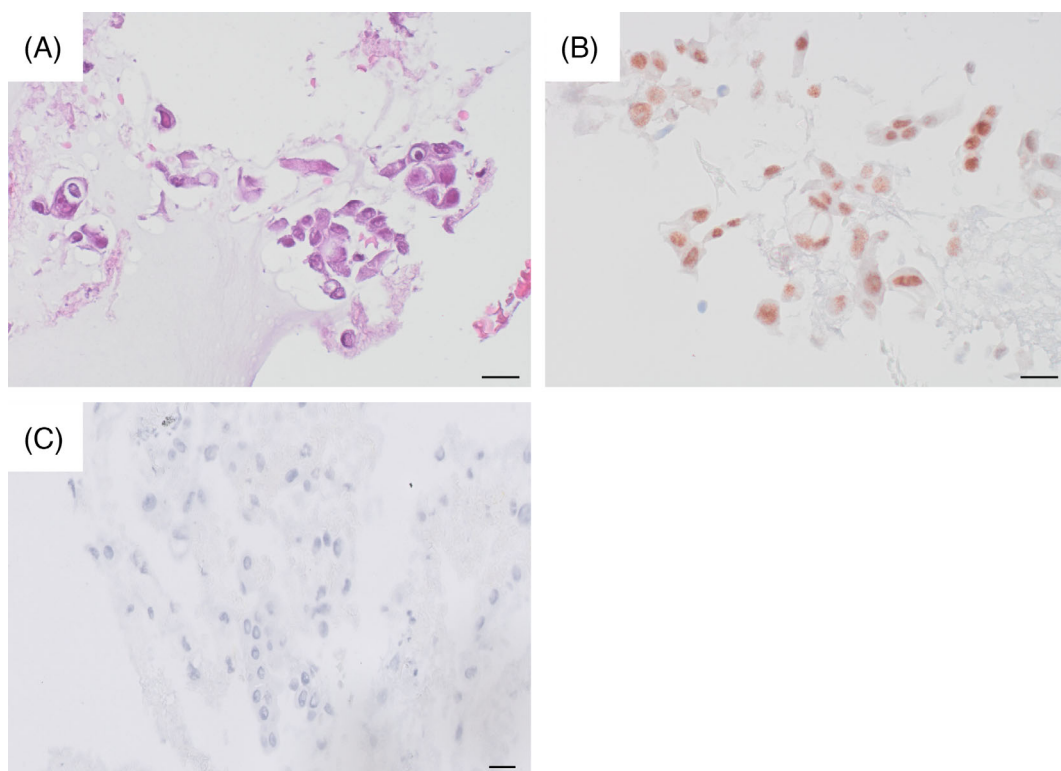


FIGURE 3 (A) Haematoxylin eosin, cell block. Atypical cells with signet-ring features and intracytoplasmic drops. Scale bar: 20 μ m. (B) Pan-TRK immunocytochemistry, cell block. Strong nuclear staining. Scale bar: 20 μ m. (C) Oestrogen receptor immunocytochemistry, cell block. Negative staining. Scale bar: 20 μ m

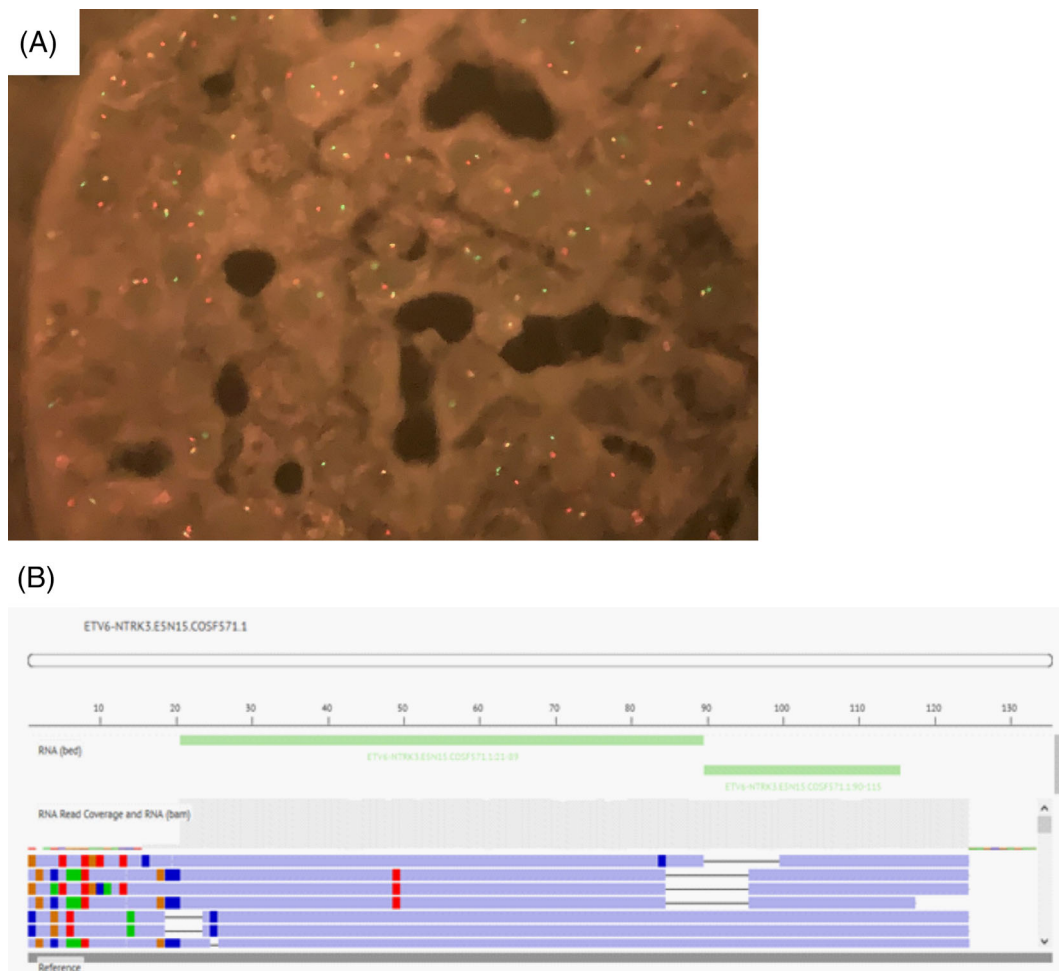


FIGURE 4 (A) NTRK3 FISH, showing the break-apart pattern of the rearrangement. (B) *ETV6-NTRK3* gene fusion proved by NGS

atezolizumab in combination with paclitaxel and bevacizumab, showing partial response after 5 cycles.

Molecular studies were carried out with immunocytochemistry on the cell block (formalin fixed and paraffin embedded) and with immunohistochemistry, FISH and NGS on the mastectomy specimen.

In the mastectomy specimen, a 2.2 cm firm poorly-delimited white mass was found. In the histopathological evaluation, we could observe that it was composed of epithelial cells arranged in a micro-cytic pattern with eosinophilic secretions in a sclerotic stroma. The cells were polygonal with eosinophilic cytoplasm and mild nuclear atypia (Figure 2A–C).

Immunocytochemical and immunohistochemical studies showed a triple negative breast tumour, negative for oestrogen receptor (EP1 clone, Agilent) (Figure 3C), progesterone receptor (PgR 1294 clone, Agilent) and HER2 (Herceptest, Agilent); and positive for GATA3 (L50-823, Roche) and Sox10 (5P267, Roche). The proliferation index (Ki67 [MIB1, Agilent]) in the surgical specimen reached 20%.

NTRK overexpression was demonstrated with immunocytochemistry against pan-TRK (1-2-3) (EPR17341, Roche) on the cell block, as well as with immunohistochemistry in the mastectomy specimen. In both cases, a striking nuclear positivity was noted (Figures 2D and 3B).

NTRK3 and *ETV6* rearrangement was proved by FISH (ZytoLight SPEC NTRK3 Dual Color Break Apart Probe (Zytovision) and LSI *ETV6* Break Apart Rearrangement Probe (Vysis); respectively). Both in the primary tumour and in the recurrence, we demonstrated *ETV6-NTRK3* (*ETV6-NTRK3.E5N15.COSF571.1*) fusion by NGS (OncoPrint™ Focus Assay (ThermoFisher Scientific), with 62,459 and 49,699 reads counts, respectively (Figure 4). FoundationOne CDx (Roche) was also performed on the mastectomy specimen, showing the *ETV6-NTRK3* fusion once again, as well as a TERT promoter substitution mutation (–124C>T) and a frameshift mutation on DNMT3A (D658fs*47). We also found variants of unknown significance on FANCA (R825G), RAD54L (P433L), IKBKE (E148_G149>DW), FGFR1 (R822C), NOTCH3 (P2209L), BRCA1 (P142H) and ALK (R806C).

3 | DISCUSSION

Secretory breast carcinoma is recognized as a distinct morpho-molecular entity due to its characteristic morphology and the presence of *ETV6-NTRK3* gene fusion.

We conducted an extensive literature review on the cytological features of secretory breast carcinoma, and we found 24 articles that

TABLE 1 Clinicopathological features of the cases included in the reviewed articles

Article	Sex	Age (years)	Size (cm)	Cytological description	Molecular testing
Craig ⁸	Woman	63	NA	Solid and papillary	NA
D'Amore et al. ⁹	Woman	48	NA	Sheets	NA
Nguyen and Neifer ¹⁰	Woman	73	2	Sheets	NA
Richard et al. ¹¹	Woman	37	1.5	NA	NA
Domínguez et al. ¹²	Woman	63	NA	Sheets and clusters	NA
Gupta et al. ¹³	NA	NA	NA	NA	NA
Shinagawa et al. ¹⁴	NA	NA	NA	Clusters	NA
de la Cruz et al. ¹⁵	NA	NA	NA	NA	NA
Pohar-Marinek and Golough ¹⁶	Man	20	NA	NA	NA
Shinagawa et al. ¹⁷	5 cases	NA	NA	Sheets and clusters	NA
Hou et al. ¹⁸	Woman	35	NA	NA	NA
Nonomura et al. ¹⁹	Woman	12	NA	Sheets and clusters	NA
Jayaram et al. ²⁰	Woman	39	1.5	Clusters	NA
Furugaki et al. ²¹	Woman	73	2.3	NA	NA
Vesoulis et al. ²²	Man	33	NA	NA	NA
Gupta et al. ²³	Woman	91	3	Sheets	NA
Gupta et al. ²³	Woman	83	4.5	Sheets	NA
Gupta et al. ²³	Woman	55	3	Sheets	NA
Gupta et al. ²³	Woman	69	6.5	Sheets	NA
Izumi et al. ²⁴	Woman	61	2.8	Single cells	NA
Alenda et al. ²⁵	Man	79	3	Sheets	NA
Oh et al. ²⁶	Woman	42	2	NA	NA
Sukpan et al. ²⁷	Woman	57	3.5	Sheets and clusters	NA
Mardi and Sharma ²⁸	Woman	52	NA	NA	NA
Jena and Shariff ²⁹	Woman	24	4	Clusters	NA
Jena and Shariff ²⁹	Woman	40	5	Clusters	NA
Shanthi et al. ³⁰	Woman	62	5	Clusters	NA
Gupta and Gupta ³¹	Woman	66	2.5	Clusters and papillae	In mastectomy specimen, technique not specified
Shukla et al. ³²	Woman	60	2.5	Clusters and papillae	ETV6-NTRK PCR in mastectomy specimen

Abbreviation: NA, not available.

described its cytological features, with a total of 33 cases. However, only in 22 studies including 25 patients, we were able to retrieve clinicopathological data. These studies included 22 women (88%) and 3 men (12%), with a mean age of 53.5 years (min 12 years; max 91 years). The tumour measurement was provided in 17 cases, with a mean size of 3 cm (min 1.5 cm; max 6.5 cm), and the final treatment was recorded in 16 patients, with mastectomy in 13 cases and lumpectomy in 3 cases.

We were able to retrieve cytological descriptions from the abstracts and/or full texts in 17 cases. Most of the smears are described as groups of epithelial cells with vacuolated cytoplasm, single cells with signet ring cell morphology and an extracellular secretion resembling globules. In just two cases *ETV6-NTRK3* gene fusion was demonstrated, although not in the cytological specimen, but in the subsequent biopsy (Table 1).

The *ETV6-NTRK3* gene fusion was first described in secretory breast carcinoma in 2002⁶ and most of the articles we found

describing its cytological features are previous to this year. Thus, to our knowledge, this is the first reported case that proves TRK overexpression, as a fusion surrogate, in the cytological sample.

The expression of NTRK by immunocytochemistry has been previously described in secretory carcinoma of the salivary gland.³³ This tumour was previously named “mammary analogue secretory carcinoma” due to its resemblance to secretory breast carcinoma, including the *ETV6-NTRK3* fusion.³⁴

SBC is associated with a favourable prognosis. However, our case has shown an atypical course, with multiple recurrences; and previous studies have reported SBC recurrences.³⁵ Moreover, metastatic SBC harbouring TERT promoter mutation, like our case, has been previously described,³⁶ and it has also been reported in metastatic and locally recurrent NTRK-rearranged thyroid carcinoma³⁷ and in high grade gliomas harbouring NTRK fusions.³⁸ Additionally, TERT promoter mutation has been the most commonly found co-alteration in a cohort of TRK fusion cancers.³⁹ Regarding DNMT3A alterations, they

have been described in metastatic breast cancer⁴⁰ and are associated with poor prognosis in papillary thyroid carcinoma.⁴¹

Although SBC is very rare, its correct diagnosis has a major impact on the management of patients with this tumour. As previously stated, it usually has a good prognosis despite showing a triple negative profile. The cytological sample might be the first diagnosis material; thus, recognition of its distinct cytological features and early demonstration of *ETV6-NTRK3* gene fusion can avoid an aggressive management. Nevertheless, the cases that do not progress well are eligible for systemic treatment with TRK inhibitors.⁴²

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Irene Carretero-Barrio, Amparo Benito. **Data curation:** Irene Carretero-Barrio, Tamara Caniego Casas, Elena López Miranda, María Eugenia Reguero-Callejas. **Funding acquisition:** José Palacios. **Investigation:** Almudena Santón. **Supervision:** Belén Pérez Mies, Amparo Benito, José Palacios. **Writing - Original draft preparation:** Irene Carretero-Barrio, Elena López Miranda, Belén Pérez Mies, Amparo Benito, José Palacios. **Writing - Review & editing:** Irene Carretero-Barrio, Almudena Santón, Tamara Caniego Casas, María Eugenia Reguero-Callejas, Belén Pérez Mies, Amparo Benito, José Palacios.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Table 1 of this article. Further information can be requested to the corresponding authors.

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