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Silibinin plus Stupp protocol as conversion therapy for unresectable glioblastoma with pSTAT3 expression, an oasis in the desert? A case report description.

Valeria Internò [a,1,*], Raffaella Messina [b,1], Luca Bertero [c], Alessia Andrea Ricci [c], Luigi Rosito [d], Ilaria Bonaparte [e], Domenico Sergio Zimatore [f], Alba Fiorentino [e,g], Camillo Porta [h,i,2], Francesco Signorelli [b,2]

[a] Medical Oncology Unit, San Paolo Hospital, Bari, Italy
[b] Division of Neurosurgery, Department of Translational Biomedicine and Neurosciences (DiBraiN), University "Aldo Moro" of Bari, Bari, Italy
[c] Pathology Unit, Department of Medical Sciences, University of Turin, Turin, Italy
[d] Operative Unit of Neurosurgery, Department of NESMOS, Sapienza University of Rome, Rome, Italy
[e] Department of Radiation Oncology, F. Miulli General Regional Hospital, Acquaviva delle Fonti
[f] Interventional and Diagnostic Neuroradiology Unit, University Hospital Policlinico of Bari, Bari, Italy
[g] Medical Department, LUM University, Casamassima, Bari, Italy
[h] Interdisciplinary Department of Medicine, University of Bari "A. Moro", Bari, Italy
[i] Division of Medical Oncology, A.O.U. Consorziale Policlinico di Bari, Bari, Italy

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ABSTRACT

Glioblastoma represents adulthood's most frequent and aggressive primary brain tumor. The gold standard in the treatment remains radical resection followed by concomitant chemo-radiation therapy according to the STUPP protocol. Despite the therapy, the prognosis remains poor. Thus, one main objective in neuro-oncology research is identifying novel therapeutic targets to improve life expectancy. In this regard, recent advances underline the importance of pSTAT3 expression in the Glioblastoma microenvironment in terms of cancer initiation and progression. Therefore, pSTAT3 inactivation due to Silibinin action may prove to be an additional therapeutic option for Glioblastoma patients. Here we report the case of a 52-year-old patient diagnosed with an unresectable right front-temporal-insular Glioblastoma by biopsy. The patient was directed upfront to concomitant chemo-radiotherapy treatment with Temozolomide and Silibinin, which had a neoadjuvant effect, obtaining an objective response that made radical surgery possible. The subsequent immunohistochemistry analyses showed a moderate expression of the signal transducer and activator of transcription 3 on reactive astrocytes surrounding tumor cells. To our knowledge, this is the first report concerning the activity of Silibinin as conversion therapy in concomitance to standard treatment and could lay the foundation for the design of prospective trials aimed at validating this scheme as a standardized neoadjuvant option for unresectable Glioblastoma patients.

1. Introduction

Glioblastoma represents the most frequent and aggressive intracranial neoplasm with an annual incidence of 3.2 per 100,000 individuals; its 5-year survival rate does not exceed 5% (Ostrom et al., 2022). Glioblastoma median overall survival (OS) reaches 18 months only in those patients treated with surgery and subsequent concomitant and adjuvant chemo-radiotherapy making Glioblastoma one of the malignant tumors with the lowest survival rate (Ostrom et al., 2022; Stupp et al., 2009). Many factors can affect Glioblastoma prognosis, one of the most important being the extent of resection (Hervey-Jumper and Berger, 2016). Hence, surgical resection plays a key role in the therapeutic management of Glioblastoma, and its extent leads to a benefit in terms of OS that is significantly longer in the case of resection than a biopsy (12.2

* Corresponding author.
E-mail address: valeria.interno@libero.it (V. Internò).
[1] Valeria Internò and Raffaella Messina are co-first authors
[2] Camillo Porta and Francesco Signorelli are co-last authors

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Vs 3.5 months, respectively) (Sanai et al., 2011). Thus, an attempt should be made, where possible, to maximize the extent of surgical resection. However, surgical resectability is limited by tumor size and site (e.g. involvement of cortico-subcortical eloquent areas, insula, internal capsule, basal ganglia and corpus callosum infiltration, bilateral extension) (Signorelli et al., 2010; Murrone et al., 2019).

In the last years, many attempts have been made to identify therapeutic interventions able to convert unresectable tumors into surgically treatable ones (Jacobo et al., 2021). Unfortunately, although few cases were reported and two underpowered clinical trials were published, a standardized and effective neoadjuvant therapy is presently not available (Kaloshi et al., 2015; Tabouret et al., 2021). Thus, a strong need exists to understand the molecular basis of this deadly disease and to develop novel therapeutic strategies that can eventually be translated from bench to bedside. Recent developments showed that aberrant activation of signal transducer and activator of transcription 3 (STAT3) may contribute to cancer initiation and progression via the promotion of cell proliferation/survival, invasion/migration, angiogenesis, and immune evasion (Poli and Camporeale, 2015; Yu et al., 2014). Furthermore, the activation of STAT3 seems to mediate tumor resistance to a broad spectrum of cancer therapies, including radiotherapy, conventional chemotherapy, and modern targeted therapies, its activation is correlated with the generation and maintenance of cancer stem cells (Zhao et al., 2016). Therefore, activation of STAT3 is a strong predictor of poor prognosis and is an independent risk factor for tumor recurrence and post-therapy progression. Accordingly, many STAT3 inhibitors (STAT3i) have been developed and among them, flavonolignan silibinin possesses drug-like properties with proven clinical activity via inhibition of STAT3 signaling (Kohsaka et al., 2012). Furthermore, it proved to be clinically useful during chemotherapy helping the liver detoxification process (Loguercio and Festi, 2011). In this paper, we describe the case of a patient diagnosed with an unresectable GBM in the right front-insulo-temporal region who received a combination of Silibinin, Temozolomide, and encephalic radiotherapy, thus achieving a tumor shrinkage that made a subsequent radical surgical resection possible. Tumor tissue obtained at surgery was examined by immunohistochemistry to assess the activation status of STAT3. The results provided novel insights into the knowledge of predictive factors for response to STAT3i

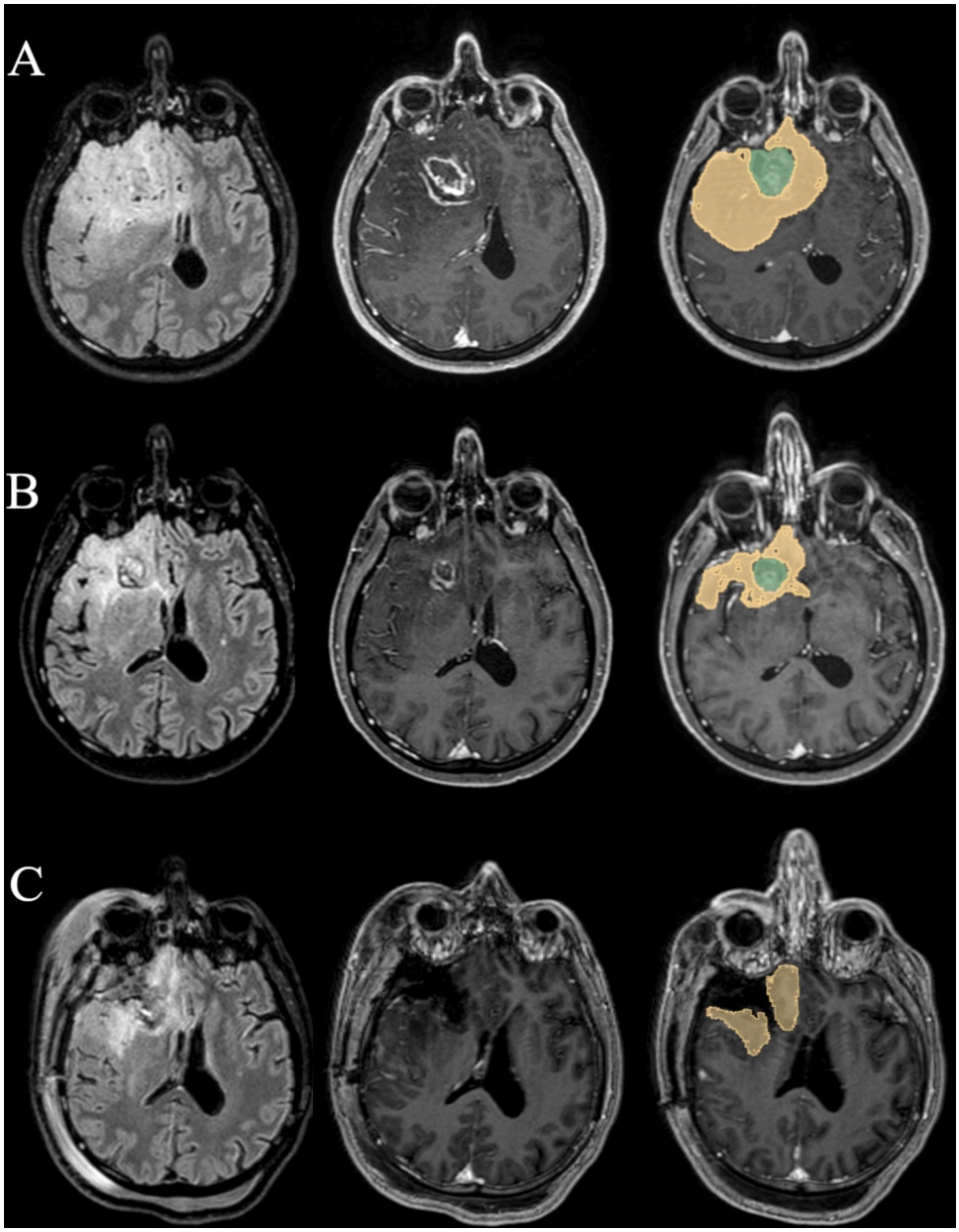

Fig. 1. Row A. MRI shows a voluminous infiltrating lesion in the right fronto-insulo-temporal site. Evidence of wide perilesional edema (FLAIR sequence, on the left); a front-basal nodule with cystic-necrotic areas in the context of the lesion (T1-weighted sequence with Gadolinium, in the middle); tumor volume before neo-adjuvant treatment (on the right). The lesion displaces the ventricular system to the left. **Row B.** MRI after neo-adjuvant treatment with Silibin. Evidence of a clear reduction in tumor volume (on the right), both in the enhancing core (T1-weighted sequence with Gadolinium, in the middle) and inner the infiltrating area, visible in FLAIR (on the left). A clear reduction of the peritumoral edema and of the dislocation of the ventricular system was also observed. **Row C.** Brain MRI was performed 24 h after surgery and documented a gross resection of the tumor.

and may help identify patients amenable to this neoadjuvant therapy.

2. Case report

2.1. Clinical presentation and diagnostic procedures

A 52-year-old man was admitted in July 2021 to the Medical Oncology Unit of the University Hospital Policlinico of Bari with a history of absence seizures leading to generalized ones. The patient underwent an encephalic MRI that showed a right fronto-temporal-insular tumor, Yasargil type 5B, Sanai giant type38,39 of 5 × 4 × 4 cm infiltrating the perisylvian opercula, the orbitofrontal and temporopolar regions, the parahippocampal gyrus and the ippocampus, with a large area of perilesional edema, a central core with inhomogeneous contrast enhancement extending into the anterior perforated substance. The ventricular system appeared compressed and slightly shifted to the right (Fig. 1, row A). The tumor volume of the enhancing core in T1-weighted images with Gadolinium was 13.95 cm^3, and the volume of the infiltrated area in FLAIR images was 182.58 cm^3. The patient's neurological status was not affected by the lesion. According to our management protocol for adults with central nervous system tumors, the patient underwent a CT-total body scan to exclude any pathological extracranial evidence and the hypothesis of metastasis.

2.2. Therapeutic process

After the multidisciplinary assessment, no indication for frontline surgical resection of the lesion was retained at first, considering the size and site of tumoral infiltration, which prevented us from a meaningful resection, equivalent to at least 90% of the preoperative tumor volume[40] as well as the high risk of postoperative neurological sequelae. Hence, a stereotactic biopsy was performed at the Neurosurgery Unit of Policlinico of Bari. The histological examination diagnosed a glioblastoma, IDH-wildtype (GBM), grade 4, pMGMT unmethylated, according to the WHO CNS tumor classification, 5th edition. Concomitant chemo-radiotherapy started 15 days after the biopsy. Radiotherapy (RT) was performed at General Regional Hospital F. Miulli, Acquaviva delle Fonti (BA). The patient, immobilized by a thermoplastic mask, received a total dose of 70 Gy in 30 fractions covering tumoral extension plus 2 mm as shown on brain T1-weighted contrast-enhanced MR images and 60 Gy in 30 fractions on tumor plus 1.5 cm of margin. RT was planned with volumetric modulated arc therapy with simultaneous integrated boost, by TrueBeam (Varian, Palo Alto, USA). Concomitant chemotherapy with radiosensitizer Temozolomide (TMZ (75 mg/m2 per day for six weeks) plus Silibinin (Sillbrain 650 mg once daily, per os) was given from day 1 to day 45. RT dose was elevated to 70 Gy with the aim of obtaining the best objective response possible considering the unresectable nature of the GBM. Brain MRI performed after treatment showed a significant reduction in the volume of the right frontal expansive lesion configuring a partial response (PR) according to the response assessment in Neuro-Oncology (RANO) criteria. The patient underwent a multidisciplinary re-evaluation, which ruled out the possibility of surgical treatment as the lesion, although reduced in size, showed still clear involvement of the nucleo-capsular region. Hence, the patient underwent 5 cycles of sequential TMZ (200 mg/m/day for 5 days/28 days) plus Silibinin and a follow-up brain MRI at seven months from biopsy demonstrated a further reduction of the extent of tumoral infiltration (82.92 cm^3 in FLAIR images) and of the enhancing core (5.02 cm^3 in T1-weighted images with Gadolinium) (Fig. 1, row B). At that time the patient was autonomous, Karnofsky Performance Status, KPS 90%. Hence, after further multidisciplinary evaluation, the patient was referred to the Neurosurgery Unit of Policlinico of Bari for surgical treatment. Microsurgical resection (KINEVO microscope with BLUE 400 and YELLOW 560 filters, Carl Zeiss Meditec, Oberkochen, Germany) was guided by neuronavigation (Stealth System S8, Medtronic, Minneapolis, MN, USA), intraoperative neurophysiological monitoring (IONM) for motor mapping and use of a weight-adjusted dose of 5-ALA (20 mg/kg body weight) (Gliolan, Photonamic GmbH Inc, Germany) and sodium fluorescein (5 mg/kg body weight) (Monico S.p.A., Venice, Italy). A gross total resection, confirmed by an early postoperative brain MRI, was achieved with no surgery-related complications (Fig. 1, row C). At the 1-year follow-up, the patient is recurrence-free and resumed his previous activities, with a KPS of 100%.

2.3. Histopathological analysis and examination of pSTAT3 expression

The histological and immunohistochemical analysis confirmed the previous diagnosis of IDH wild-type, pMGMT non-methylated GBM. Further immunohistochemical investigations were performed on the resection sample at the University of Turin: immunohistochemical (IHC) horseradish peroxidase DAB staining using the anti-pSTAT3 (Tyr705) D3A7 XP antibody (Cell Signaling, Danvers, MA, USA) was used to assess the activation status of STAT3 and GFAP IHC alkaline phosphatase Fast Red staining (anti-GFAP SP78 antibody, Roche Molecular Systems, Pleasanton, CA, USA) was performed to stain neoplastic cells and reactive astrocytes. Within the tumor core, pSTAT3 expression was restricted to few glioblastoma cells showing faint to moderate nuclear staining (Fig. 2, A-B). A higher rate of positive pSTAT3 neoplastic cells was observed in tumor infiltration areas just outside the tumor core (Fig. 2, C-D), but they remained a minority of all neoplastic cells. Finally, rare pSTAT3-positive putative reactive astrocytes were observed in infiltrated brain parenchyma far from the tumor core (Fig. 3, A-B).

3. Discussion

The standard upfront therapy for Glioblastoma is represented by maximal safe surgical resection followed by concomitant chemo- and radiotherapy according to the protocol recommended by Stupp et al., followed by adjuvant chemotherapy with sequential TMZ (Stupp et al., 2009). Modern advances in neurosurgical techniques, with the introduction of IONM, neuronavigation, intraoperative imaging, and fluorophores widened surgical indications and improved resection rates and prognosis of these patients (Picart et al., 2017). Moreover, progresses in adjuvant treatment concur in prolonging survival and quality of life for newly diagnosed and relapsing Glioblastoma.

However, unresectable Glioblastoma patients retain the poorest prognosis. For such tumors, the treatment is confined to concomitant chemo- and radiotherapy, and the only attempt to maximize the objective response, although not a standardized approach, is limited to an increased radiation dosing. Apart from anecdotic reports, neoadjuvant treatment is not included in the neuro-oncological armamentarium for Glioblastoma (Tabouret et al., 2021). On this account Silibinin, a natural plant component of milk thistle seeds known to potentiate toxic effects of chemotherapy drugs such as TMZ with a minimum rate of side effects and whose mechanisms of action involve inhibition of STAT3 signaling can be considered a valuable option for this purpose. In fact, several recent studies demonstrated a positive correlation between histopathological grade and the extent of STAT3 activation and showed constitutive activation of STAT3 in 66–83% of Glioblastoma (Kohsaka et al., 2012). Our case report for the first time shows a radiologically relevant response to the concurrent administration of STUPP protocol with enhanced RT and silibinin in a case of primary Glioblastoma initially deemed inoperable and the focal pSTAT3 expression evidenced in the resection sample could be related to the pharmacological inhibition of pSTAT3 obtained through the Silibinin treatment. This treatment regimen acted as neoadjuvant therapy and promoted subsequent gross total resection, unfeasible at diagnosis, with an improvement in survival and quality of life compared to STUPP protocol after a simple biopsy. Therefore, due to the low response rate usually obtained after standard treatments, the high response rate obtained by our patient makes it possible to confer Silibinin an important additive effect, although only prospective trials will confirm this

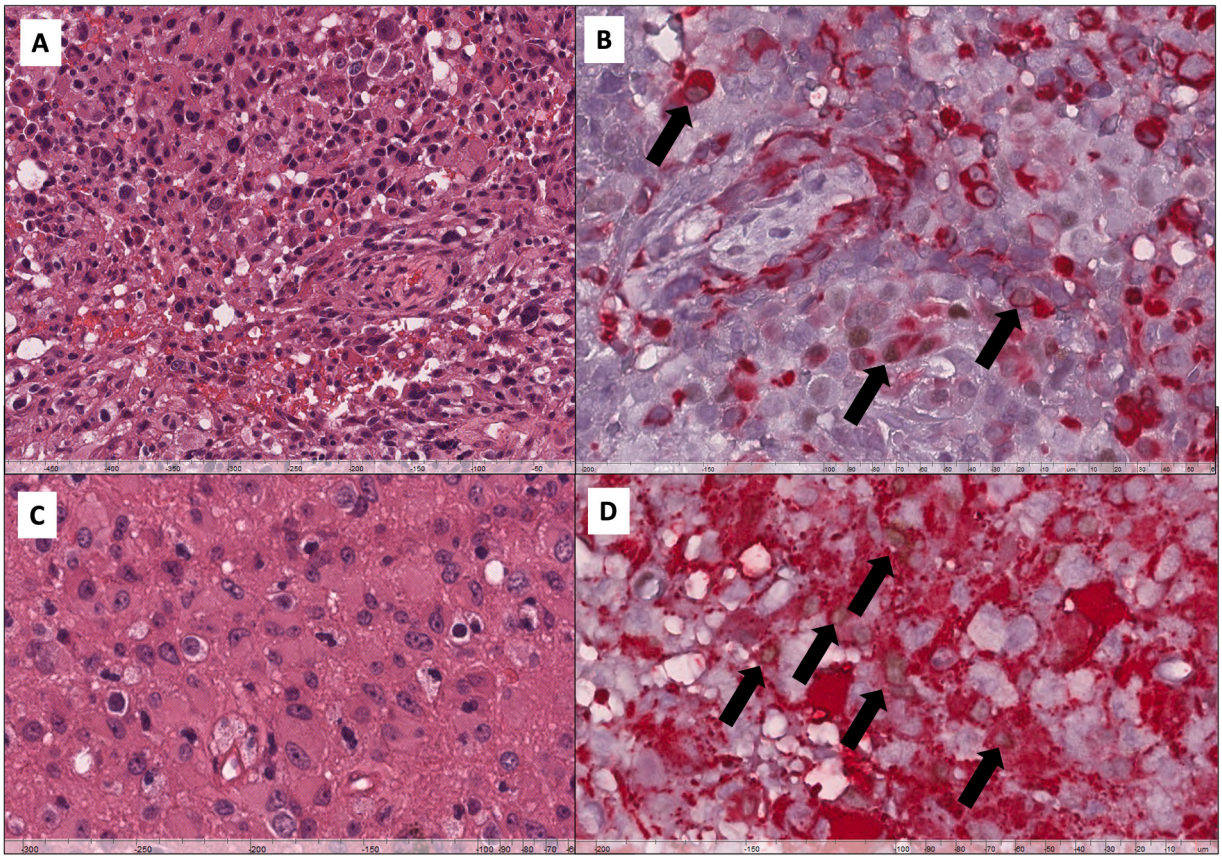

Fig. 2. Within the tumor core, pSTAT3 expression was restricted to few glioblastoma cells showing faint to moderate nuclear staining (**A-B**). A higher rate of positive pSTAT3 neoplastic cells was observed in tumor infiltration areas of adjacent brain parenchyma (**C-D**), but they remained a minority of all neoplastic cells.

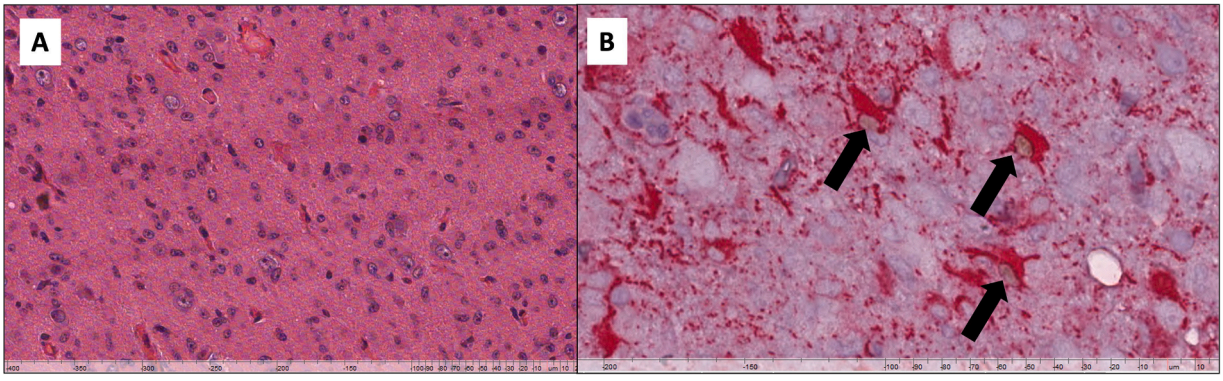

Fig. 3. The assessment of reactive astrocytes is more challenging in diffuse gliomas compared to brain metastases due to the marked infiltration of adjacent brain parenchyma which makes more challenging the distinction between reactive and neoplastic cells. Nevertheless, it was possible to observe rare pSTAT3-positive putative reactive astrocytes in infiltrated brain parenchyma (**A-B**).

hypothesis. The level of expression of pSTAT3 does not seem to be a limiting factor, as in our case it was moderate. A possible explanation is that the examination has been made in the surgical specimen, after Silibinin treatment and so, the moderate expression could reflect the response to anti-pSTAT3 treatment. Otherwise, only prospective trials will define how and where p STAT3 expression have a predictive role of response to Silibinin.

4. Conclusion

Concomitant use of flavonoid therapy with silibinin and STUPP protocol may act as neoadjuvant treatment, with the aim of reducing tumor infiltration, thus allowing gross radical resection for Glioblastoma deemed inoperable at diagnosis. The safety profile of silibinin and the level of expression of pSTAT3 do not seem to be limiting factors and may allow for expanded surgical indications, portending an improvement in prognosis. However, only prospective trials will confirm the efficacy of this new scheme of conversion therapy for Glioblastoma.

Patient consent statement consent to publish

The patient in this report provided consent for the anonymous publication of her/his experiences. Written informed consent for publication of his/her case was obtained from the patient.

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CRediT authorship contribution statement

Valeria Internò: Conceptualization, Methodology, Investigation, Writing – original draft. **Raffaella Messina:** Conceptualization, Methodology, Investigation, Writing – original draft. **Luca Bertero:** Formal analysis. **Alessia Andrea Ricci:** Formal analysis. **Luigi Rosito:** Resources, Visualization. **Ilaria Bonaparte:** Visualization, Project administration. **Domenico Sergio Zimatore:** Visualization, Project administration. **Alba Fiorentino:** Visualization, Project administration. **Camillo Porta:** Writing – review & editing, Supervision. **Francesco Signorelli:** Conceptualization, Methodology, Investigation, Writing – original draft.

Declaration of Competing Interest

None.

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