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EDITORIAL

RE-STARTING FROM A SOCIAL-ECOLOGICAL APPROACH TO HEALTH

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COVID-19: CRISIS AND OPPORTUNITY

The Covid-19 pandemic, being an "extraordinary" event, has led to a serious crisis of the "ordinary": of medicine (and its established certainties) and of the healthcare system (and especially its sclerotic practices).

The emergency has hit our lives like an unexpected storm. Not because a pandemic was not expected - pandemics are cyclical - but because the power of science and technology had given us an illusion of invincibility. The very idea of death seemed to have been dismissed, banished, to some extent defeated. As Gordon pointed out, medicine had offered us the illusion that «humans can overcome nature, no longer a victim, but in the omnipotent driver's seat» (1).

Death and illness, however, have returned with force to mark our days through the bulletins that arrive from the territories and inform us of new cases, admissions to hospital and intensive care, and deaths. The Covid-19 pandemic reminded us that «the radical autonomy projected in western society is a social construct, aided greatly by naturalism and biomedicine» (1).

As to healthcare organisation in Italy, the pandemic has highlighted the limits of a structure of services focussed on highly technological hospital care, rather than on primary care, and in general too much oriented towards therapy and very little towards prevention activities. The pandemic has brought to the forefront the central role of communities - meant as groups of people who live or work together, or who share relationships, interests, and habits - and of community institutions (families, associations, informal networks, *etc*.), in taking care of patients. On the whole, we can say that Covid-19 has highlighted the limits of an approach to care and health that may go unnoticed by those who are generally in good health and come into contact with the service system in a sporadic and occasional manner, but that has already negatively affected those categories of people who were in a particularly fragile condition: the chronically ill, the elderly, immigrants and ethnic minorities, the homeless. And it pointed the way to reorient the health system from "cure" to "care".

HEALTH AS QUALITY OF THE "BETWEEN"

We have clearly seen how the effects of Covid-19 are influenced by an altered human-environment balance as much as by a deterioration in the human-human relationship within societies, where the weight of inequalities is still heavy.

As to the first issue, several studies seem to show that a correlation exists between short-term exposure to atmospheric pollutants and the spread of COVID-19 (2-4). For example, Pozzer *et al*. estimated that, on average, about 15% of all deaths caused by Covid-19 worldwide are attributable to long-term exposure to air pollution, and this percentage increases further in some countries (29% in the Czech Republic, 27% in China, 26% in Germany, 22% in Switzerland) due to low air quality caused by the presence of fossil fuels (5).

The plausibility of causal links between pollution, contagiousness and symptomaticity of SARS-CoV-2 would call into question fine dust (especially PM 2.5 particulate matter), which seems to play a role in inducing the over-production, by the cells of the respiratory mucosa, of ACE2 receptors (the same receptors that act as a gateway to the virus) (5-7).

As to the second issue, chronic diseases typically associated with poverty and socio-economic disadvantage increase the severity and lethality of the infection. Specifically, a vicious circle is observed between chronic diseases and Covid-19: chronic diseases increase the clinical severity of Sars-CoV-2 infection, and the infection exacerbates pre-existing clinical conditions in carriers of comorbidities (asthma, COPD, obesity, hypertension, diabetes, *etc*.). This connection seems to highlight the role of social inequalities in determining an impact of the disease on the population. It is no coincidence that, with reference to Covid-19, Horton revived the expression "syndemic" created by the anthropologist Merrill Singer to describe and explain the correlation between the various morbid conditions (such as non-communicable diseases and infectious diseases) and the socio-economic and environmental interacting factors that amplify the negative effects on health (8-9). At the same time, the pandemic has contributed to exacerbate inequalities, that have impacted on the management of the emergency, marking a significant difference between those who could choose to stay at home and those who could not, those who could isolate themselves from people, and those who lived in promiscuous places and could not avoid infection (10-12).

The pandemic has therefore powerfully re-proposed a "socio-ecological" concept of health: no longer merely understood as a condition internal to living beings (the proper functioning of the body-machine), but as the quality of the "between", *i.e*. of the relationships that bind us to the natural environment and the social fabric, and which prove capable of conditioning the quality of life and well-being of people. The pandemic reminded us that "the human body is not a machine, that health and illness are not merely biological states but rather that they are conditions which are intimately related to and constituted by the social nature of human life" (13). As Didier Fassin pointed out, people are unequal in the face of illness and death due to the material conditions of their existence, which have an influence on their state of health as well as on their ability to care for themselves. In this way, differences in status and wealth are inscribed in bodies, converting "*le social en biologique*" (14).

But how can we rebuild from the lessons learned from Covid-19, and from this systemic vision of health? What are the implications in terms of health policies? How to re-start?

THE TWO FRONTS OF THE RESTART

Two fronts seem to open up, one which is strictly related to the environment, and the other one related to the remodelling of health services and activities.

Environment and health

On the first front, there is the need to rebuild the relationship with the environment, in terms of greater salubrity and sustainability. To this purpose, the Mission 6 - Health of the Italian National Recovery and Resilience Plan (hereinafter NRRP) envisaged a new governance system to redefine prevention strategies and interventions in the health, environmental and climate fields, and the way health needs related to pathologies with environmental aetiology are addressed (15). The aim is to enhance the advocacy role and capacity of the Italian National Health System in intersectoral actions (according to the "health in all policies" approach), by creating a new National System for Health-Environment-Climate Prevention − in synergy with the current actions for the environmental protection coordinated

by the Ministry of the Environment. This new System will focus on: monitoring and controlling the effects of environmental contamination on health; managing health risks of environmental origin; and building decision-making scenarios, according to a transdisciplinary, multi-institutional and cross-sectoral approach, which connects diverse fields (economic development, mobility, urban planning, use of land and water, agriculture, safety in relation with energy choices and the green transition, digital and technological developments, *etc*.) (16).

The NRRP reform action is connected also with the Investments Plan proposed in the "Complementary Fund" financed through the multi-year budget variance approved by the Italian Council of Ministers. These investments converge on two main lines: on the one hand, the overall strengthening of the structures and services of the National System for Health-Environment-Climate Prevention at national, regional, and local level; on the other hand, the development of specific operational programs aimed at experimenting, in selected contaminated sites, models of "ecological public health" (17) informed by the principles and guidelines of health-environment-climate integration. This latter aspect, which is particularly innovative, aims to combine - within an integrated and systemic approach - the actions of environmental detection and bio-monitoring (to support the identification of the pollutants, of the effects on health in terms of genetic and epigenetic alterations, and of individual susceptibility), with interventions of primary prevention (risk mitigation and minimization through environmental remediation and requalification), secondary prevention (active health surveillance) and organization of health care (development of diagnosis, treatment and rehabilitation paths).

Health services: from medical deserts to proximity

In terms of services, health care can only become more responsive to the real needs of people and more equitable in granting access to care for all if it restarts from the understanding of the dialectic between health needs, healthcare supply and demand, with reference to the increasingly widespread concept of "medical desertification". The term "medical desert" does not refer only to the simple "absence" of services, but also to the poor quality and low accessibility of health care paths. This analysis, functional to the reprogramming of healthcare services, must be developed with specific attention for those fragile groups that require a greater protection effort from the service system, in particular the chronically ill, the elderly, migrants and ethnic minorities, women (with reference to the gender issue) and socio-economically disadvantaged groups, for whom the risk of suffering the negative effects of an inaccessible service system is higher (18).

The analysis should take place along three fronts, in line with the framework proposed by the WHO (19). The first front is that of availability. When considering a territory, the first question is: *are there services?* In other words, it is necessary to assess whether the number of health professionals, the supply of beds, territorial facilities, residential facilities, home care programs are sufficient in relation to the distribution of the population and its specific epidemiological characteristics.

The second aspect is that of quality: *the services are there, but are they working?* As a matter of fact, healthcare practices sometimes lack efficacy and services do not always guarantee appropriate standards of care, from a clinical and organizational point of view.

Finally, there is a third question: *the services are there and are working, but do they work for each and everyone in the same way?* This question leads to the great issue of equity, which involves the accessibility as well as the acceptability of treatments, also in relation to the values and the preferences of the patient. As a matter of fact, in some circumstances, legal, economic, social, linguistic-cultural, logistical, organizational barriers may determine inequalities in access to healthcare.

From an operational point of view, the key word in health planning papers seems to be "proximity": an expression associated with positive meanings and values capable of supporting action.

Proximity healthcare is integrated healthcare organized on a local scale, easily accessible and therefore permeable, which "looks out" from institutional spaces to intercept emerging needs and dialogues with civil society, the care resources that come from the territories (private social organizations, patient associations, neighbourhood communities) and other public entities (research, environment, social sector). In this approach, the paradigm of "waiting for" is replaced by "going towards", to reach and enter the silence that often surrounds those who experience situations of greater discomfort.

The interventions that support a remodelling in the perspective of proximity fall into three strategic macro-areas: a) outreach activities, *i.e*. socio-health activities carried out in places close to the commu-

nities and with easy access or directly in the living and working places of the target groups (for example, active offer of first and second level services and screening programs through the use of mobile clinics, or home care), in which operators are asked to leave traditional health facilities to reach those who would otherwise experience difficulty in accessing treatment, or would not be able to express a request for help; b) system mediation, which includes measures and initiatives aimed at improving the accessibility and usability of traditional health services. Examples for this include: involving case-managers with the role of facilitators within health facilities; adopting agile and "low-threshold" organizational solutions, including the creation of integrated clinical-assistance paths for specific typologies of patients to promote appropriateness and continuity of care, but also to reduce slowness and the indifference of bureaucratic mechanisms in the face of the urgency of illness and discomfort; planning and implementing training plans for operators on issues related to relational and communication aspects; and c) active involvement of target groups, *i.e*. strategies aimed at creating resilient communities by enhancing the role of the single individuals and community institutions (such as families, associations, informal networks, *etc*.) who are involved - in agreement and/or in synergy with the health and social care operators - in the design, implementation and evaluation of actions for the promotion and protection of health (20).

A SYSTEM PERSPECTIVE

The crisis triggered by the COVID-19 pandemic offers us the opportunity to reflect - in this time of change that preludes to a change of times - on the evolution of the concept of health and on the implications that this entails in terms of promotion, prevention, and protection of the individual and collective well-being.

It becomes crucial to adopt a systemic, anti-reductionist, multidisciplinary and intersectoral perspective which, in line with the Declaration of Alma-Ata, considers health in relation to the material, biological, cultural, and social dimensions of life and fosters the development of effective policies and actions for each of these dimensions.

To make a significant contribution to health improvement, all public health interventions and strategies should be included within intersectoral programs that should take into account the socially produced conditions and dynamics, that interact with the biological and environmental factors, contributing to influence the health-disease processes.

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The authors have declared no conflict of interests.

Authors' contribution

All the authors contributed equally to conception, data collection, analysis and writing of this paper.

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RESEARCH ARTICLE

AIOM RECOMMENDATIONS ON THE USE OF CYTOREDUCTIVE SURGERY AND HIPEC IN PRIMARY AND SECONDARY PERITONEAL TUMORS

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ABSTRACT

Peritoneal surface malignancies represent rare and hard-to-treat entities that include primarily abdominal that involve disseminating cancer cells into abdominal peritoneum, with or without associated extraperitoneal disease. Diagnosis and management of these aggressive cancers need a dedicated multidisciplinary team and a high-volume center for locoregional treatment where technically and clinically feasible.
This article summarizes the most updated evidence-based guidelines that the Italian Medical

Oncology Association (AIOM) has implemented with a multidisciplinary panel of experts, including dedicated expert clinicians such as pathologists, surgeons, medical oncologists, and the support of methodologists, to guide clinicians involved in the primary management of patients with peritoneal neoplasms in their daily clinical practice. Based on the type of studies addressing the questions and their methods, AIOM guideline methodologists used the GRADE method (Grading of Recommendations Assessment, Development, and Evaluation: GRADE) to classify the quality of each kind of evidence.
In selected cases, the main curative treatment consists of a cytoreduction surgery (CRS) that implies the complete removal of the macroscopically appreciable disease or any minimum residual millimeter. It is then associated with intraperitoneal chemo-hyperthermia (HIPEC), carried out at the end of the surgical demolition time, a particular type of chemotherapy that exploits the combined effect of heat and high concentrations of drugs, with a localized action in the area affected by the neoplasm.
We here provide recommendations for 6 main clinical scenarios: primary treatment of primary serous peritoneal papillary carcinoma, pseudomixoma peritonei, colorectal and gastric cancer, ovarian carcinoma, and peritoneal mesothelioma.

KEY WORDS

Peritoneal tumors; cytoreductive surgery; HIPEC; guidelines.

IMPACT STATEMENT

This paper represents a synthesis of 2021 clinical practice guidelines about presentation, diagnosis and management of primary and secondary peritoneal surface malignancies provided by an expert panel on behalf of AIOM.

INTRODUCTION

Peritoneal surface malignancies represent rare and hard-to-treat entities that include primarily abdominal (*e.g.*, appendiceal neoplasms or peritoneal mesotheliomas) or secondary tumors (from abdominal or gynaecological cancers) that involve the dissemination of cancer cells into abdominal peritoneum, with or without associated extraperitoneal disease. Ovarian cancer, mesotheliomas, primary appendiceal carcinomas, and other primary abdominal carcinomas (colorectal, gastric, or pancreatic) may cause peritoneal carcinomatosis. In such cases, the main clinical sign is malignant ascites: the accumulation of fluid results from blockage of the draining lymphatic channels (which generally keep the amount of intraperitoneal fluid low) and increased vascular permeability. Advanced cancer with peritoneal carcinomatosis may also cause in later stages diarrhea, constipation, nausea, abdominal pain, bloating, weight loss or gain, loss of appetite, or early gastric fullness.
In 2018 the Italian Medical Oncology Association (AIOM) published the first edition of specific clinical practice guidelines for primary and secondary peritoneal tumors (1), which were subsequently updated in 2020. This article summarizes the most updated evidence-based guidelines that the AIOM has implemented with a multidisciplinary panel of experts, including dedicated expert clinicians such as pathologists, surgeons, medical oncologists, and the support of methodologists, to guide clinicians involved in the primary management of patients with peritoneal neoplasms in their daily clinical practice. Based on the type of studies addressing the questions and their methods, AIOM guideline methodologists used the GRADE method (Grading of Recommendations Assessment, Development, and Evaluation: GRADE) to classify the quality of each kind of evidence. In particular, the GRADE method assesses methodological bias within the studies, uniformity between different studies results; consistency of results across different studies; repeatability of results on a broader patient sample set; the effectiveness of treatments. Treatment comparisons result in one out of four GRADE scores, reflecting the quality of the

STRONGNESS OF CLINICAL RECOMMENDATION	TERMS	MEANING
STRONG POSITIVE	Strong Positive "In patients with (selection criteria) the xxx intervention should be considered as a first intention therapeutic option".	The intervention in question should be considered as the first therapeutic option (evidence that the benefits outweigh the harm).
WEAK POSITIVE	"In patients with (selection criteria), the xxx intervention can be considered as a first intention therapeutic option, as an alternative to yyy".	The intervention in question can be considered as a first intention option, aware of the existence of alternatives equally feasible (uncertainty regarding the prevalence of benefits over damages).
WEAK NEGATIVE	"In patients with (selection criteria), the xxx intervention should not be considered as a first intention treatment option, as an alternative to yyy".	The intervention in question should not be considered as a first intention option; it could, however, be suitable for use in highly selected cases and after complete sharing with the patient (uncertainty regarding the prevalence of harm over benefits).
STRONG NEGATIVE	"In patients with (selection criteria), the xxx intervention must not be considered as a first intention therapeutic option".	The intervention in question must in no case be taken into consideration (evidence that the harm prevails over the benefits).

Table I. *Strongness of clinical recommendation graded in four levels based on clinical relevance.*

evidence: high-quality, moderate-quality, low-quality, or very low-quality evidence. The strength of the recommendation is graded, based on clinical importance, on four levels (**table I**).

The patient's clinical history greatly conditions the diagnostic classification of peritoneal neoplasms. Specifically, the cases in which a peritoneal neoplastic pathology is diagnosed during the follow-up of a known primary neoplasm should be distinguished from those in which the patient's medical history is mute. In the first case, the diagnostic process aims to verify the correlation between the metastatic event and previous cancer. In the second case, however, the diagnostic workout must be planned according to the invasiveness of the procedures and resources availability.

The diagnostic process must first consider the more frequent pathologies according to the sex and age of the patient. The other diagnostic elements that emerge from clinical evaluations, blood chemistry, and instrumental tests (tumor markers, computed tomography, PET-CT, MRI, endoscopies) must be integrated with the epidemiological data. The indication for the surgical, diagnostic procedure (laparoscopy or exploratory laparotomy) must be placed with extreme accuracy and if the other diagnostic procedures could not lead to the diagnosis of certainty (**figure 1**). These invasive procedures allow both the biopsy of neoplastic material and an accurate estimate of the extent of peritoneal disease using the Peritoneal Cancer Index (PCI). PCI is a value determined by the size of the peritoneal implants and the distribution of nodules on the peritoneal surface. For the evaluation of the final score, the size of the peritoneal nodules is first assessed; The sum of the lesion size score and the distribution of tumor in the abdominopelvic regions gives us the patient's PCI. Implants are scored as lesion size 0 through 3 (LS-0 to LS-3). LS-0: no implants are seen throughout the region; LS-1: implants visible up to 0.5 cm in greatest diameter; LS-2: nodules greater than 0.5 cm and up to 5 cm; LS-3: implants 5 cm or greater in diameter (2). However, it is not recommended to proceed with aggressive surgical attempts and debulking before a complete and correct diagnostic classification. Adverse events resulting from improper abdominal-pelvic surgical manipulation are worrisome. They include disseminating neoplastic cells in the surgical explored areas that remain trapped in fibrosis and fibrin and are may become poorly sensitive to systemic and local-regional chemotherapy treatments, inevitably leading to cell proliferation and neoplastic growth (3-7).

Cytoreduction surgery (CRS) implies the concept of surgical radicality with the complete removal

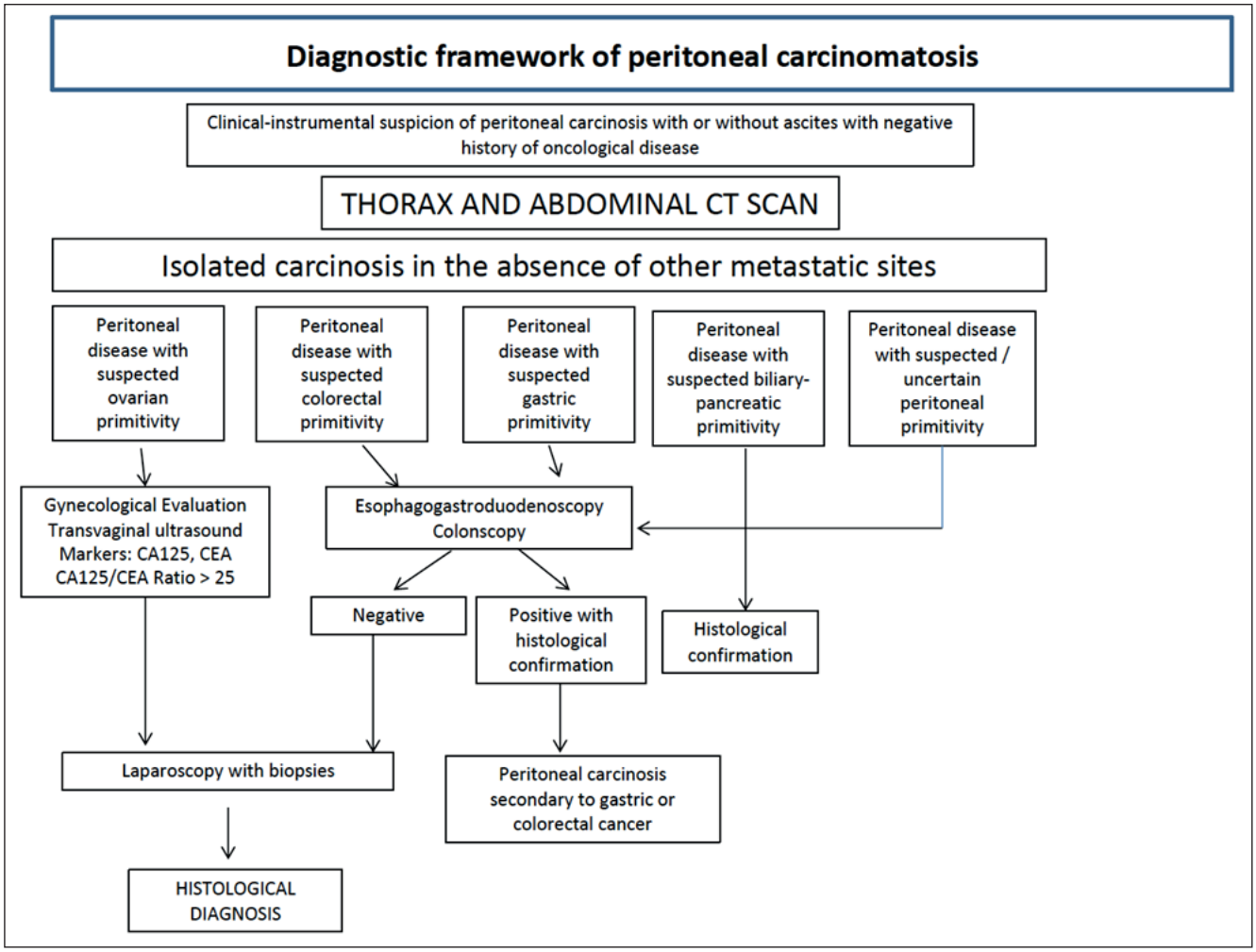

Figure 1. *Diagnostic workup of peritoneal carcinomatosis.*

of the macroscopically appreciable disease or any minimum residual millimeter. This concept differs from debulking, which implies palliative removal of part of the neoplastic disease with gross neoplastic residue. CRS for peritoneal tumors is a concept developed by Paul H. Sugarbaker (8). The completeness of Cytoreduction (CCR) is coded at the end of the surgical phase according to the criteria validated by the Consensus Conference of 2006 (9, 10). It represents the most important prognostic factor of peritoneal neoplasms and is expressed by cc-score: CC-0 (absence of visible residue), CC-1 (residue lower than 2.5 mm), CC-2 (residue greater than 2.5 mm and less than 5 cm), CC-3 (residue greater than 5 cm and with confluent nodules).

Intraperitoneal chemo-hyperthermia (HIPEC), carried out at the end of the surgical demolition time, represents a locoregional therapeutic aid, supplementary to the surgical intervention; its action is manifested in the moment of maximum cytoreduction of the neoplasm, in the absence of adhesions, and above all before the cells released in the abdomen are implanted on the bloody surfaces or are protected by the physiological deposition of fibrin and stimulated to proliferation by the chemical mediators of inflammation. It is a particular type of chemotherapy that exploits the combined effect of heat and high concentrations of drugs, with a localized action in the area affected by the neoplasm. It consists in the perfusion of the abdominal cavity with a variable quantity (3-6 liters) of liquid (Perfusate) in which high doses of chemotherapy are administered in conditions of hyperthermia. HIPEC is performed immediately after finishing the CRS by placing a system of cannulas through the abdominal wall connected to an extracorporeal circulation circuit. Therefore, the perfusate is circulated with chemotherapeutic agents in conditions of hyperthermia with a closed abdomen or an open abdomen. Duration of perfusion, type of chemotherapy and temperatures are a function of the histological type to be treated (11, 12).

RECOMMENDATIONS FOR CRS AND HIPEC

In patients with Primary Serous Peritoneal Papillary Carcinoma (SPPC), the neoadjuvant chemotherapy followed by HIPEC associated with CRS is indicated compared to chemotherapy treatment followed by interval debulking surgery? (table II)

SPPC is histologically similar to epithelial ovarian cancer (EOC) but clinically differs by a predominantly peritoneal widespread, with a little ovarian involvement. The epidemiological, clinical and molecular differences between SPPC and EOC have been highlighted and described in a review (13).
The exact incidence of SPPC is not clear, and actually, about 10-20% of EOC labeled as serous papillary ovarian carcinoma are SPPC.
Due to the similarities with EOC, SPPC has often been treated by surgery and systemic chemotherapy (sCT) containing platinum and taxanes. Therefore, a lot of data arises from small retrospective cohorts or case-control, comparing patients with SPPC and EOC. The median overall survival (mOS) of patients with SPPC is 21-42 months, shorter than EOC patients, with a progression-free survival (PFS) of 11-17 months (14-16).
The experience gained in other peritoneal neoplasms through CRS and HIPEC has motivated various groups to extend the indications also on SPPC as a primary peritoneal neoplasm. The rationale for this approach is based on the multifocality, polyclonality, and the high frequency of widespread peritoneal metastases of SPPC. The analysis of 36 patients with SPPC treated with CRS and HIPEC was conducted in France and Italy (17). In addition, 35 patients received platinum-based systemic adjuvant treatment. Morbidity and mortality were 20.6% and 5.6%, respectively. Five-year OS was 57.4% and DFS was 24% (median of 16.7 months).
A single-center analysis of 29 patients with SPPC homogeneously treated with neoadjuvant chemotherapy with 6 cycles of Carboplatin and Paclitaxel followed by CRS and HIPEC, after a median follow-up of 12 months, showed a 5-years OS of 64.9% (median not reached) (18, 19). Overall, grade III-IV surgical complication was seen in 4/22 (18%) patients; no post-operative mortality was observed. Median PFS was 32.9 months, and 5-year PFS was 33.2%. CRS was performed with total parietal peritonectomy and with HIPEC using a chemotherapy combination based on Cisplatin plus Doxorubicin at 43 Celsius degree of temperature for 90 minutes.
Based on these results, the absence of randomized studies comparing the integrated treatment to standard chemotherapy and surgical debulking limits the significance of the results. However, the benefit assessed as overall and progression-free survival of sCT plus CRS and HIPEC treatment and relatively limited adverse events compared to sCT and surgical debulking, get the judgment in favor of the benefit over the damage, but it must be discussed with the patient regarding the extension of the surgical treatment. Therefore, the panel provided a positive recommendation in favor of sCT plus CRS and HIPEC.

In patients with resectable pseudomyxoma peritonei, is HIPEC associated with cytoreduction indicated rather than surgical debulking and systemic chemotherapy?

The main supporting literature included the McBride, *et al*. study (20), which is a review and meta-analysis of 15 observational studies concerning the treatment of pseudomyxoma peritonei by CRS associated with HIPEC in various forms (EPIC, HIPEC, HIPEC + EPIC). Median survival at 3, 5, and 10 years was 77.85%, 79.5%, and 55.9%, respectively. The median complication rate (calculated across 14 studies) was 40%. Although the complication rate is not negligible, the panel believes that the benefit given by CRS associated with HIPEC is still higher than that provided by repeated debulking surgeries.
Based on these assessments and experience in the field, the AIOM panel unanimously judged the balance between risks and benefits deriving from the execution of CRS and HIPEC in resectable pseudomyxoma peritonei to be favorable and provided a weak positive recommendation in favor of intervention.

If operability and resectability criteria are met in patients with diffuse malignant epithelioid peritoneal mesothelioma, are cytoreductive and HIPEC procedures indicated compared to systemic chemotherapy?

In patients with peritoneal mesothelioma, the efficacy of the combined treatment with CRS and HIPEC is reported in numerous papers reported in the literature (21-24). In summary, the results offered by palliative chemotherapy alone with convention-

DISEASE	GRADE QUESTION	STAGE	TIMING	DRUGS USED	RECOMMENDATION
PRIMARY SEROUS PERITONEAL PAPILLARY CARCINOMA (SPPC)	In patients with SPPC, the neoadjuvant chemotherapy followed by HIPEC associated with CRS is indicated compared to chemotherapy treatment followed by interval debulking surgery?	III	After 3-6 cycles of systemic chemotherapy	Cisplatin	Weak positive
PSEUDOMYXOMA PERITONEI	In patients with resectable pseudomyxoma peritonei, is HIPEC associated with cytoreduction indicated rather than surgical debulking and systemic chemotherapy?	Intraperitoneal metastases (intra-abdominal disease only)	Upfront	Various*	Weak positive
PERITONEAL MESOTHELIOMA (EPITHELIOID HISTOLOGY)	In patients with diffuse malignant epithelioid peritoneal mesothelioma, if operability and resectability criteria are met, cytoreductive and HIPEC procedures are indicated compared to systemic chemotherapy?	All stages (operable stage I-II disease or stage III after primary CT)	Upfront	Cisplatin-based*	Weak positive
OVARIAN CANCER (HIGH-GRADE SEROUS HISTOLOGY)	In patients with high-grade serous ovarian carcinoma in stage IIIC who have received neoadjuvant chemotherapy, cytoreduction with HIPEC and further chemotherapy (3 cycles) should be considered compared to cytoreduction alone after systemic chemotherapy?	IIIC	After 3 cycles of systemic chemotherapy	Cisplatin	Weak positive
COLORECTAL CANCER	In patients with colorectal carcinoma and synchronous or metachronous peritoneal carcinosis, PCI < 16, favorable biology, and good general condition, cytoreduction and HIPEC should be considered compared to systemic therapy?	IV (with peritoneal carcinomatosis)	Upfront of after 4-6 cycles of systemic chemotherapy	MMC (preferred) or platinum-based regimens*	Weak positive
GASTRIC CANCER	In patients with gastric carcinoma and only synchronous peritoneal metastasis, PCI < 6, ECOG performance status 0-1, and a therapeutic response after a first-line treatment, cytoreduction and HIPEC should be considered versus systemic chemotherapy?	IV (with peritoneal carcinomatosis)	After 4-6 cycles of systemic chemotherapy	Various*	Weak negative

Table II. *Clinical recommendations for CRS and HIPEC.*

al agents (pemetrexed and cisplatin) are extremely disappointing (median OS < 8-10 months). In a systematic review including 20 observational studies, Helm et al. analyzed a total of 1,047 patients with peritoneal mesothelioma with median PCI of 19 (25). An optimal intervention of CRS (complete cytoreduction-0/1) + HIPEC was performed in 67% of patients. Overall survival at 1, 3, and 5 years was respectively 84, 59, and 42%, being significantly higher than historical data with traditional treatment. Deraco, *et al*. reported an overall and progression-free 5-year survival of 57% and 31%, respectively, in a single-center series (26). The postoperative grade 3 morbidity was 15% in the absence of mortality correlated with the surgery, while the toxicity resulting from the chemo-hyperthermic treatment was 12%. Surgical radicality, performance status, and mitotic counts were statistically correlated with the results. In a further monocentric experience reported by Robella et al. (27), the OS at 1 and 5 years was 63% and 44%, respectively, with an overall morbidity rate of 35.7% associated with a perioperative mortality of 7.1%.

Regarding the quality of life after HIPEC CRS treatment, the potential high morbidity correlated with the complexity of the surgical procedure must be taken into account. In the experience of Piso et al. (28), even if the published data show a compromise in the postoperative quality of life at three months after surgery, there is subsequently an improvement over 6-12 months to levels higher than baseline. The evidence of the results relating to survival and quality of life despite the frequency of adverse events allowed the panel to unanimously judge a positive balance between benefits and risks deriving from the execution of CRS and HIPEC and recommended in favor of intervention.

In patients with high-grade serous carcinoma of the ovary in stage IIIC who have received neoadjuvant chemotherapy, should CRS + HIPEC and further chemotherapy (3 cycles) be considered an alternative to CRS alone after systemic chemotherapy?

The Netherlands Cancer Institute conducted a large, randomized, open-label phase III study by Van Driel et al. Their results were published in the New England Journal of Medicine in January 2018 (29). The trial was conducted on 245 patients with stage III serous ovarian cancer and included, after three courses of neoadjuvant chemotherapy with carboplatin and paclitaxel, a 1: 1 randomization with CRS only (n = 123 patients) versus CRS plus HIPEC with Cisplatin 100 mg/m2 alone (n = 122 patients). Subsequently, adjuvant treatment with systemic chemotherapy was delivered in both groups for three cycles with carboplatin and paclitaxel. The primary endpoint was relapse-free survival (RFS). The median RFS was 10.7 months in the surgery-only group versus 14.2 months in the CRS plus HIPEC group. At a median follow-up of 4.7 years, mortality was higher in the CRS group (62% of patients) than in the CRS and HIPEC group (50% of patients) (hazard ratio, 0.67; 95% CI, 0.48 to 0.94; p = 0.02). The median survival, secondary endpoint, was higher in the experimental CRS plus HIPEC group (45.7 months) than in the CRS alone group (39.9 months). The percentage of grade 3-4 adverse events reported in the two groups was almost overlapping, respectively 25% in the CRS group and 27% in the CRS plus HIPEC group (p = 0.76), supporting the feasibility and tolerability of the integrated procedure.

In consideration of the available data, AIOM judged favorable the balance between risks and benefits and provided a positive recommendation for the execution of CRS associated with HIPEC and subsequent systemic chemotherapy (3 cycles) after disease control with neoadjuvant chemotherapy in patients with high-grade, stage III, serous ovarian carcinoma. However, in patients eligible for this approach, such treatment should be carried out at high-volume centers where high expertise is expected.

Should CRS and HIPEC be considered the only alternative treatment to systemic therapy in patients with colorectal carcinoma and synchronous or metachronous peritoneal carcinosis, PCI < 16, favorable biology, and good general condition?

In a small open-label randomized Swedish study by Cashin et al. published in the European Journal of Cancer in 2016 and closed prematurely for poor accrual, 48 patients were randomized to receive CRS plus HIPEC with 5-FU versus oxaliplatin-based chemotherapy alone (30). Median survival was 25 *vs*. 18 months in favor of the experimental arm (RR 0.79, 95% CI 0.64-0.97) with a 21% reduction in the absolute risk of death. However, the progression-free survival (PFS) was lower in magnitude (RR 0.83, 95% CI 0.7-1). Against these efficacy data, no fatal events occurred at 30 days (strong evidence). 12 serious adverse events were reported in 10 patients in the experimental arm, compared with 14 grade 3-4 events reported in 12 patients in the

chemotherapy arm, with overall low evidence of consistency of the toxicity.
For this reason, the panel unanimously judged favorable the balance between risks and benefits deriving from the execution of CRS and HIPEC in peritoneal carcinosis from synchronous or metachronous colorectal carcinoma PCI < 16, favorable biology and good general conditions and provide a weak positive recommendation in favor of intervention.

Should CRS plus HIPEC versus systemic chemotherapy be used for patients with gastric carcinoma and only synchronous peritoneal metastasis, PCI < 6, ECOG performance status 0-1, and a therapeutic response after a first-line treatment?

Peritoneal carcinomatosis of gastric origin is recognized as an independent poor prognostic factor associated with poor prognosis. Systemic chemotherapy options do not differ from those of metastatic disease, although carcinosis is a factor associated with poor response to systemic treatment, mainly due to poor bioavailability of drugs on the peritoneal surface. According to the current state of evidence and literature data, although it represents a field of research in high-volume centers, the locoregional treatment with CRS and HIPEC in gastric adenocarcinoma with peritoneal carcinomatosis does not seem to represent a recommended treatment at least in the Western population. Although one randomized study (31) demonstrates a non-significant trend in favor of higher five years OS, the confidence intervals are broad and similar results are also reported for PFS. The panel, therefore, believes that the expected desirable effects (prolongation of OS and RFS / PFS) resulting from the integrated treatment of CRS and HIPEC in the PS 0-1 patient with peritoneal carcinosis alone from primary gastric cancer and a PCI < 6, are negligible and so provide strong negative recommendation against it.

ETHICS

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The authors have declared no conflict of interests.

Availability of data and material

N/A

Authors' contribution

All the authors contributed equally to conception, data collection, analysis of this paper. AD and FP wrote this article.

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N/A

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REVIEW

THE USE OF GRANULOCYTIC COLONY-STIMULATING FACTORS IN PATIENTS RECEIVING CHEMOTHERAPY FOR GERM CELL TUMORS

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ABSTRACT

Germ cell tumors (GCT) are a relatively rare malignancy occurring in adolescent/young males in the vast majority of cases. With the advent of platinum containing regimens 4 decades ago, impressive cure rates have been achieved both in the adjuvant setting and in first line for advanced disease, with the vast majority of patients being cured. Moreover, 35 to 50% of patients with recurrent disease can be cured with conventional on high-dose chemotherapy programs. Neutropenia and its complications (mainly neutropenic fever) may be a limiting toxicity with the risk of reducing the planned curative doses or delaying intervals with potential impact on outcome. Granulocytic-hematopoietic growth factors (G-CSFs) by limiting neutropenia and neutropenic fever allow the delivery of curative chemotherapy in a safer manner to GCTs patients. We will present an overview of the use of G-CSF in this setting.

KEY WORDS

Granulocytic colony-stimulating factors; chemotherapy; germ cell tumors; G-CSF prophylaxis; bleomycin.

IMPACT STATEMENT

The proper delivery of chemotherapy in germ cell tumors is crucial to achieve excellent results in terms of cure. G-CSFs may allow the clinicians to deliver chemotherapy in a timely manner and to reduce the risk of severe neutropenia and neutropenic fever.

INTRODUCTION

Germ cell tumors (GCTs), represent a minority of solid tumors, and they are the most frequent occurring in men aged 15-40 years (1, 2). They are histologically divided into two major categories; seminoma and non-seminoma (3). GCTs have been the first solid tumors to achieve impressive high cure rate with first line chemotherapy; but even in second and subsequent lines, the disease is often curable (4-6). According to the recent IGCCCG Update Consortium report, patients with metastatic seminoma show 5-year overall survival (OS) of 95% and 88% in good and intermediate prognosis groups, respectively (7). These figures are better than those presented in the IGCCCG classification published in 1997 (8). In non-seminoma, clinical improvement is even higher regarding poor prognosis patients: OS increasing from 48% to 67% in the last two decades, with a minor, but clear better outcome also for good (from 92% to 94%) and intermediate prognosis groups (from 80% to 89%) (9). Such impressive cure rates are mainly related to the introduction of cisplatin-based chemotherapy at the end of the '70s. The combination of cisplatin, etoposide, bleomycin (BEP) has proved to be superior in terms of outcome and toxicity compared to PVB (cisplatin, vinblastine and bleomycin) (10). BEP still represents the backbone of first line therapy in metastatic GCT after nearly 35 years from its introduction. The successful story in our ability to cure young patients with GCT, other than advances in systemic and local treatment modalities including expert surgery, derives from the promotion of multidisciplinary team-based care that ensures greater adherence to clinical guidelines and other components of quality of care, such as reducing treatment toxicity and minimizing delays in diagnosis and greater utilization of surveillance (4, 11). Chemotherapy regimens employed in GCTs may induce non-negligible adverse effects which can compromise the high cure rates of the treatment itself. In order to minimise morbidity, mortality and to maintain this high-cure rate, neutropenia and related complications are hurdles to be removed on the pathway to cure. We will comment on the role of these complications in the treatment of GCT and the role of granulocyte colony-stimulating factors (G-CSFs).

FEBRILE NEUTROPENIA IN GERM CELL CANCER PATIENTS

Febrile neutropenia (FN) is defined as an oral temperature of > 38.3 °C or two consecutive readings of > 38.0 °C for 2 hrs and an absolute neutrophil count (ANC) of < 0.5 × 10^9/l, or expected to fall below 0.5 × 10^9/l (12); it may be a life-threatening complication of platinum-based chemotherapy (13).

Although the occurrence of FN depends on several factors including chemotherapy regimen, dose intensity, host performance status, previous treatment(s) and bone marrow function, it is difficult to predict exactly the risk for a certain schedule in a single patient. Scoring systems have been published to identify such risk and they may be helpful to the clinicians in order to evaluate the prognosis of an infectious event during febrile neutropenia (14).

The impact of FN from the socio-economic aspect is also to be taken into consideration in terms of lengths of hospitalization and burden of costs (15, 16).

As most GCT patients are young, with adequate bone marrow function and few comorbidities, the risk of neutropenia largely depends on the chemotherapy schedule itself in this population.

Chemotherapy for advanced disease in germ cell patients has a curative intent in most cases and toxicity-related changes in the planned dose and schedule may have a detrimental effect on outcome (17).

In the early trails with BEP or PVB, grade 4 neutropenia was recorded in nearly 60% of patients, and 2.5 % developed fatal sepsis (10).

While BEP is the standard upfront chemotherapy regimen, second and subsequent lines, often given with curative intent, include cisplatin plus ifosfamide and/or paclitaxel and/or vinblastine, and even high-dose chemotherapy with stem cell support (5, 6, 18). All these regimens have a potential high rate of neutropenic fever (19, 20).

In the pre- G-CSF era a retrospective study in the UK (21) evaluated the incidence of neutropenic fever in 88 patients undergoing 240 courses of BEP or CEB (carboplatin replacing cisplatin) and receiving or not receiving prophylactic ciprofloxacin at the dose of 250 mg bid at the onset of neutropenia grade 3. Neutropenic fever was recorded in 5% of patients receiving ciprofloxacin compared to 15% of those not receiving prophylaxis. Neutropenia grade 3 or 4 was recorded among patients receiving BEP in 65%.

In a series from Graz University (22) FN was reported in nearly 17% of 413 consecutive patients; in a multivariate analysis, adjusted for age and risk classification, revealed that poor performance status, seminomatous histology and prior radiation therapy were associated with an increased risk of FN.

The advent of G-CSFs has given us a formidable tool for reducing the risk of NF and its consequences in the treatment of solid tumors (23).
Currently available G-CSFs include short-term G-CSF preparations (filgrastim, lenograstim), requiring daily administrations, and long-acting preparations that they only need to be administered once following chemotherapy (pegfilgrastim and lipegfilgrastim). Both originators and biosimilars have the same efficacy in reducing the risk of FN and related complications (24, 25).

G-CSF PROPHYLAXIS FOR CHEMOTHERAPY-RELATED NEUTROPENIA IN GERM CELL TUMORS

In a randomized EORTC study including 120 poor-prognosis advanced testicular cancer treated with standard BEP or intensified BOP/VIP, G-CSF primary prophylaxis improved the delivery of planned treatment schedule, and reduced the toxic death rate in the intensification arm (19).
In a nationwide retrospective analysis conducted by the National Cancer Institute in Slovakia the use of primary prophylaxis with G-CSF in patients receiving BEP showed statistically significant reduction in the rate of febrile neutropenia (10% with G-CSF *vs* 32% in patients not receiving G-CSF) (26).
Major international guidelines recommend the use of G-CSFs as primary prophylaxis in patients undergoing chemotherapy with a predictive risk of febrile neutropenia around 20% and above (27).
Several of the most employed regimens in the treatment of advanced GCTs have such a potential (24), so the use of G-CSF is highly advisable with the aims of avoiding prolonged and profound neutropenia as well as maintaining dose intensity and timing. In fact, as these tumors are curable with standard multiple-drug regimens, less myelosuppressive agents/regimens are not available with the same efficacy (**table I**).
In second or subsequent chemotherapy lines the incidence of hematologic complications is high.
In a reported series from Memorial Sloan-Kettering Cancer Centre 46 patients were treated in second line (32) with four courses of TIP (paclitaxel, ifosfamide and cisplatin); all received G-CSF 5 ug/kg daily from day 7 until day 18. The rate of febrile neutropenia was high (48%) and the progression free survival at more than 5 years was 65%.
One of the more recent schedules employed in third line is TPG (paclitaxel, cisplatin and gemcitabine) (33) developed at the NCI in Milan with the three drugs administered a week apart (day 1 and 8). G-CSF was planned daily on day 9. Febrile neutropenia was recorded in 7% of the courses and nearly 30% of the patients developed grade IV neutropenia.

High-dose chemotherapy

In the last three decades high-dose chemotherapy (HDC) supported by peripheral blood progenitor cells have become an option for recurrent GCTs.

AUTHOR (REFERENCE)		CHEMOTHERAPY REGIMEN	PRIMARY G-CSF PROPHYLAXIS	FEBRILE NEUTROPENIA	GRADE IV NEUTROPENIA
Williams SD, 1987	(10)	PVB/BEP	No	NR	59%
Fosså SD, 1998	(19)	BOP/VIP-B	No Yes	46% 25%	49% 18%
De Wit R, 1998	(28)	VIP	No	11%	26%
Bathia S, 2000	(29)	HDCT	Yes	51%	100%
Hinton S, 2003	(30)	VIP	No	8%	70%*
Kondagunta GV, 2005	(5)	TIP	No	48%	130**
Culine S, 2007	(31)	3 BEP 4 EP	36% 29%	7% 5%	72% 90%
Kondagunta GV, 2007	(32)	HDCT	Yes	67%	100%
Necchi A, 2014	(33)	TPG	No	7%	29%
Terbuch A, 2018	(22)	Mainly BEP	14.9%	16.9%	NR

Table I. *Risk of neutropenia and febrile neutropenia of different chemotherapy regimens in germ cell tumors.*
PVB: cisplatin, vinblastine, bleomycin; BEP, bleomycin, etoposide, cisplatin; NR, not reported; BOP/VIP, bleomycin, vincristine, cisplatin/vinblastine, ifosfamide, cisplatin; HCDT, high dose chemotherapy; TIP, paclitaxel, ifosfamide, cisplatin; EP, etoposide, cisplatin; TPG: paclitaxel, gemcitabine, cisplatin.
*Grade IV hematologic toxicity; **median neutrophils count at nadir/microliter.

Curves plateauing around 40% have been published even in third line (4, 15) and many guidelines suggest this therapy as a possible option (27, 34).
G-CSFs are crucial in the mobilization phase of peripheral blood progenitor cells (usually at 10 ug/kg daily). In the event of poor mobilization new agent plerixafor has been added to G-CSF with excellent results (35). G-CSF reduces the length of neutropenia allowing faster recovery following HDC; in this setting single dose of pegfilgrastim can be used replacing several injections of daily G-CSF filgrastim (36).

SPECIFIC SETTINGS

Elderly patients

As mentioned before, GCTs are diseases of adolescents and young adults and the incidence after the age of 50 or 60 years is rare, with only 5-8% of patients included in this age category (37, 38).
Incidence of hematologic complication, in particular FN, in this older population is higher compared to the younger counterpart (33). The use of G-CSF has been suggested to be mandatory as primary prophylaxis in this population, as 44 percent of the patients developed > 1 episode of NF (39). In the previously cited study by Terbuch *et al.* (22) G-CSF was recommended in patients over 50 years of age due to higher risk of neutropenia-related complications. In the very few patients older than 75 years reported in the literature, full dose BEP can be safely delivered with G-CSF prophylaxis (40) as reported in other more common diseases (41).

Bleomycin and G-CSF

Bleomycin is still nowadays a cornerstone of first line regimen in germ cell tumors. It is well known that the administration on this drug can result in the serious complication of pulmonary fibrosis probably due to the lack of the enzyme bleomycin hydroxylase in the lungs. The incidence of this event has been reported as high as 8% in patients exposed to > 300 IU with a mortality rate of 1% to 3% (42).
As standard BEP for four courses includes 90 IU each cycle, a careful pre and on therapy check of respiratory function tests is highly advisable. Early studies with bleomycin and G-CSF did not suggest that G-CSF is causally related to an increase in bleomycin pulmonary toxicity also in patients treated for Hodgkin disease (43). What is important is a possible development of renal damage due to cisplatin which can lead to increased bleomycin lung toxicity. In a retrospective series of 212 patients treated at the Peter McCallum Cancer Center in Melbourne, the rate of bleomycin inducing pneumonitis was 34%, the majority being asymptomatic (only radiological findings). In this series the use of G-CSFs (either daily G-CSF or pegfilgrastim) was not randomized. The use of G-CSFs did not have a significant effect on the severity of bleomycin lung damage (44).
A recent Canadian report (45) on 88 patients, treated with germ cell tumors and Hodgkin disease with or without filgrastim (in a not randomized fashion), adds further evidence that the concomitant use of filgrastim does not increase the risk of pulmonary toxicity of bleomycin (45). Another topic regards the best timing for G-CSF administration in BEP schedule**.** BEP regimen includes a second and third administration of bleomycin (30 IU) on days 1, 8 and 15 or 2, 9 and 16. As G-CSFs have not to be administered within the 24-hour period prior to chemotherapy due to the schedule of this regimen, G-CSFs can be started on day 6, but soon interrupted, despite the fact that bleomycin is not a myelotoxic drug.
Possibilities to overcome this hurdle is to deliver peg-G-CSF either on the day 6 anticipating the second dose of bleomycin on the day 5 or starting daily G-CSFs on day 10 until day 15. Another option developed at the European Institute of Oncology in Milan is to deliver bleomycin 15IU as an intravenous push on day 1 and 10 IU i.v. continuous infusion over 12 hours on days 1 to 3. In their experience on 182 patients the efficacy of this modified BEP regimen was comparable to standard BEP (46), allowing G-CSFs to be administered after completion of CT until neutrophil recovery.

CONCLUSIONS

Due to high cure rate of GCTs, it is mandatory to deliver the planned treatment schedule of chemotherapy. For this reason, primary and secondary G -CSF prophylaxis should be considered, in accordance with the recommendations of the main scientific societies, for patients with GCTs undergoing chemotherapy both at conventional doses (*i.e.*, ifosfamide-containing) and within intensified/HDC programs. In selected clinical situation, including fragile/elderly patient, G-CSF should also be given when chemotherapy is administered with a curative intent.

ETHICS

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There were no institutional or private fundings for this article.

Conflict of interests
The authors have declared no conflict of interests.

Availability of data and material
No new data were generated or analysed in this research.

Authors' contribution
All the authors contributed equally to conception, data collection, analysis and writing of this paper.

Ethical approval
N/A

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ORIGINAL ARTICLE

CYTOMATRIX, A NEW PROCEDURE TO ENHANCE THE DIAGNOSTIC USEFULNESS OF FINE NEEDLE ASPIRATES

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ABSTRACT

Fine-needle aspirates are still the basis of cytodiagnosis for neoformation of visceral organs; nevertheless, there is an increasing role for ancillary testing. Specimens obtained are not always optimal, and prevent a conclusive diagnosis when other complementary tests are needed to reach this goal. This study aims at evaluating a novel technology called "CytoMatrix" to entrap the cytology collections in a synthetic matrix that can be processed as a histology specimen, allowing to perform immunohistochemical and molecular biology analyses. Cytological material from twenty-five fine-needle aspirates were collected from different anatomical areas and from benign and malignant lesions. The collected aspirated material was transferred onto CytoMatrix, and processed for histology, immunohistochemistry and FISH analyses. In all the cases processed with the synthetic matrix, final diagnosis was reached. Nevertheless, immunohistochemistry and FISH were successfully performed on the malignant neoformations analyzed and the data produced allowed to precisely define the phenotype of the cancer. The results show that this synthetic matrix allows an easy and fast analysis of morphological and molecular characteristics of fine-needle aspirate material from various lesions.

KEY WORDS

Fine-needle aspirate; cytology; immunohistochemistry; breast cancer; FISH.

IMPACT STATEMENT

Definition of an original synthetic matrix that allows an easy and fast analysis of morphological, immunohistochemical and molecular characteristics of fine needle aspirate material from various malignancies.

INTRODUCTION

In the panorama of diagnostics of any type of superficial or deep neoformation, cytology has always been considered less reliable than histology. Considered a very simple method, with little risk for the patient and almost no impact on the lesion, it is unfortunately in many cases not very exhaustive, with the need for further study with histology (1, 2). The reasons are many: from the lack of representation of the structure in which the sampling is carried out (cell types are recognized but not their organization), to the difficult standardization of the fixing and sampling procedures, in addition to the specific dependence of the sampler. Nevertheless, the fact that once fixation and staining have taken place, the material is no longer usable for further diagnostic investigations, is a significant limitation (3-6).

MATERIAL AND METHODS

Cases enrolled

Twenty-five fine needle samples were performed on neoformations of various organs, in detail parotid, breast, skin and lymph nodes. Cases were enrolled randomly. The samples were collected with a 5 ml syringe mounting a 27-gauge needle, by applying negative pressure. Written informed consent was obtained from all the subjects before the collection of the fine needle samples with CytoMatrix. This device was developed and patented by UCS Diagnostic S.r.l. (Pub No. WO2018083616. International Application No: PCT/IB2017/056812). For all cases, classic histopathology analysis was accomplished on tissues obtained either through surgical excision or tru-cut biopsy.

CytoMatrix methodology

Fine needle aspirates were moulded on the CytoMatrix sponge. This synthetic matrix is made up of chitosan, a biocompatible material characterized by high ion affinity for cell samples. Chitosan has the capability of efficiently entrapping very small amounts of biological material taken up by needle aspirates, such as single cells or microscopic cell aggregates , inside its three-dimensional structure. The sponge was, then, inserted into a plastic bio-cassette and handled with the steps of classical histological technique. In detail, the aspirated complex CytoMatrix-material was processed as follows: fixation in formalin for at least 12 hours; processing, paraffin embedding and microtome sectioning; application to the sections obtained, of the various diagnostic techniques used in the histopathology laboratory, such histological staining, immunohistochemistry and FISH

Histological staining, immunohistochemistry and FISH

Paraffin sections obtained from the cytological material entrapped in CytoMatrix were cut at 5 µm using a microtome LEICA SM 2000R (Advanced Research Systems Inc., Macungie, PA), dewaxed in xylene, rehydrated through a series of graded ethanol solutions and stained with Gill's Haematoxylin and Eosin (Bio-Optica, Milan). Immunohistochemistry was executed on an automated immunostainer (Bond-III, Leica, Biosystems, Italy), as previously described (10). The primary antibodies used were respectively: Estrogen receptor (clone 6F11), Progesterone receptor (clone 16), ki67 (clone MM1), c-erbB-2 (clone CB11) and E-Cadherin (clone 36B5) (Leica, Biosystems, Italy). Images were obtained by using a light microscope (Microscope Nikon ECLIPSE 55i) equipped with a Digital Image Capture software (Leica Application Suite V4.8). FISH was performed in breast cancers showing 2/3 + HER2 immunohistochemical protein expression using a HER2/CEN 17 dual-color probe kit (ZytoLight Spec, Bremerhaven, Germany). Each slide was observed by using a fluorescence microscope (Eclipse e1000-Nikon) to evaluate the signal of hybridization. A signal ratio of HER2 to chromosome 17 was recorded in a count of a minimum of 50 tumor

cells and a ratio of ≥ 2 was regarded as HER2 gene amplification. Breast cancer with HER2 amplification was taken as a positive control.

RESULTS

Twenty-five fine needle samples were executed on neoformations of various organs, in particular parotid, breast, skin and lymph nodes. Standard hematoxylin and eosin stains were performed, together with analyses by immunohistochemistry and molecular biology studies (FISH). Successively, histological analysis was always performed on tissues obtained either through surgical excision or tru-cut biopsy. **Table I** summarizes the different samples enrolled in this study, as well as the analyses performed. In detail, immunohistochemistry was carried out on all cases of breast malignancy through E-Cadherin, Estrogen and Progesterone receptors, Ki-67, and Her2-neu antibodies. Finally, in four cases with 2/3 + HER2 immunohistochemical protein staining, also FISH analysis was performed. The Cytomatrix processing method made it possible a final diagnosis in all cases examined. There were no inadequate or doubtful cases and histological analysis on tissues obtained by surgical excision or tru-cut biopsy confirmed the diagnostic data obtained with CytoMatrix. Finally, all the breast cancer samples collected with the synthetic matrix, were appropriate for immunohistochemical and FISH analysis, thus allowing the complete immunophenotypic and molecular description of the tumours. Nevertheless, also for immunohistochemistry and FISH analysis there was full agreement with the data obtained with the classic histological sampling. In **figure 1** a paradigmatic example of histological and immunohistochemical staining in a ductal carcinoma performed on paraf-

SITE	CYTOMATRIX DIAGNOSIS	HISTOLOGICAL DIAGNOSIS	IHC	FISH
Sub-cutis	Keratin cyst	Keratin cyst		
Parotid gland	Warthin's tumor	Warthin's tumor		
Parotid gland	Pleomorphic adenoma	Pleomorphic adenoma		
Parotid gland	Pleomorphic adenoma	Pleomorphic adenoma		
Lymph node	Lymphoadenitis	Lymphoadenitis		
Lymph node	Lymphoadenitis	Lymphoadenitis		
Lymph node	Breast cancer metastasis	Breast cancer metastasis		
Lymph node	Breast cancer metastasis	Breast cancer metastasis		
Breast	Ductal carcinoma	Ductal carcinoma	Done	Done
Breast	Ductal carcinoma	Ductal carcinoma	Done	Done
Breast	Ductal carcinoma	Ductal carcinoma	Done	Done
Breast	Ductal carcinoma	Ductal carcinoma	Done	Done
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Ductal carcinoma	Ductal carcinoma	Done	
Breast	Fibroadenosis	Fibroadenosis		
Breast	Fibroadenosis	Fibroadenosis		

Table I. *Characteristics of the patients enrolled and immunohistochemical and FISH data performed.*

fin sections obtained from the cytological material entrapped in CytoMatrix is depicted. In **figure 2**, the overlapping histological and immunohistochemical results of the same ductal carcinoma after surgical resection are presented. **Figure 3** shows the results of FISH analysis on four cases with 2/3 + HER2 immunohistochemical protein staining: three out of four cases displayed HER2 amplification. Finally, in **figure 4** the histological aspect of the material collected by CytoMatrix from a fine needle aspirate of a neoformation of the parotid gland is depicted.

DISCUSSION

Fine-needle aspiration is considered a not invasive, and easy to perform procedure to diagnose pathologies in various anatomical sites. In the last years with the significant step forwards in the knowledge of the molecular mechanisms causing cancer as well as other pathologies, there has been a significant increase in the requests of biochemical and molecular information in the cytodiagnosis with the final intent to better determine treatment and prognosis (11). Regrettably, cytology specimens, especially once the material has been fixed and stained for the observation at the microscope, often are not suitable for such analyses both for the quality and the quantity of the biological material (2, 3). Moreover, performing other cytological samplings may be not possible. Finally, a significant number of ancillary techniques on tissues have been settled to work on material fixed in formalin and paraffin-embedded. This is the case, for example, for immunohistochemistry and FISH (12).

In the scientific literature there is a plethora of cell block techniques that have been defined in order to solve these problems. Goal of this article is not to make a comparative analysis between these techniques and CytoMatrix, but to describe the novelty of this procedures and some preliminary data about its reliability.

The use of the synthetic matrix in collecting the cytological sampling allows, on the one hand to recover a significant amount of good quality cytological material and on the other, applies the same methods of histology for the final preparation of the histological preparation. Last but not least, the method allows to recover and observe under the microscope not only single cells, but also microscopic fragments of tissue that preserve, at least in part, the histological structure. This very often facilitates diagnosis. For this reason, we propose the term "micro-histological diagnosis" for this procedure. It is also necessary to consider that, since the biological material is included in paraffin, it is possible to carry out several consecutive sections, on which to perform different histological staining and/or immunohistochemical and FISH analysis techniques

The Cytomatrix method is easy to apply, is reliable, but above all it can be of great help in those cases in which histological sampling is impractical or not recommended. This method encompasses all these areas: from the simplicity of sampling with a fine needle, to the specificity of the structure representation, to the possible use of the material taken for all further diagnostic investigations (histochemistry, immunohistochemistry, molecular biology). This method can be used not only to make a diagnosis (benign or malignant lesion), which is necessary for an improvement in the diagnostic and therapeutic process, but can be used in cases where histological sampling (as well as surgery) it is not recommended (severe cardiac, hematological and/or vascular pathologies) or impossible due to patient situations (advanced age, severe disability, multi-organ failure). In these cases, a simple sampling with a fine needle will be able to pick up suitable material on which it will be possible not only to make a diagnosis with hematoxylin and eosin staining, but with the material taken it will be possible to perform all the histochemistry, immunohistochemistry and also molecular biology tests. Data produced in this article support the idea that pathologists could include CytoMatrix method, among the various techniques adopted to investigate cytological specimens, to make the cytological material appropriate not only to morphological analysis, but also to immunohistochemical and molecular analysis. Further experimentations are ongoing to demonstrate the suitability of the cytological material collected with this technique for more sophisticated assays of molecular biology, such as mutational analysis and next generation sequencing.

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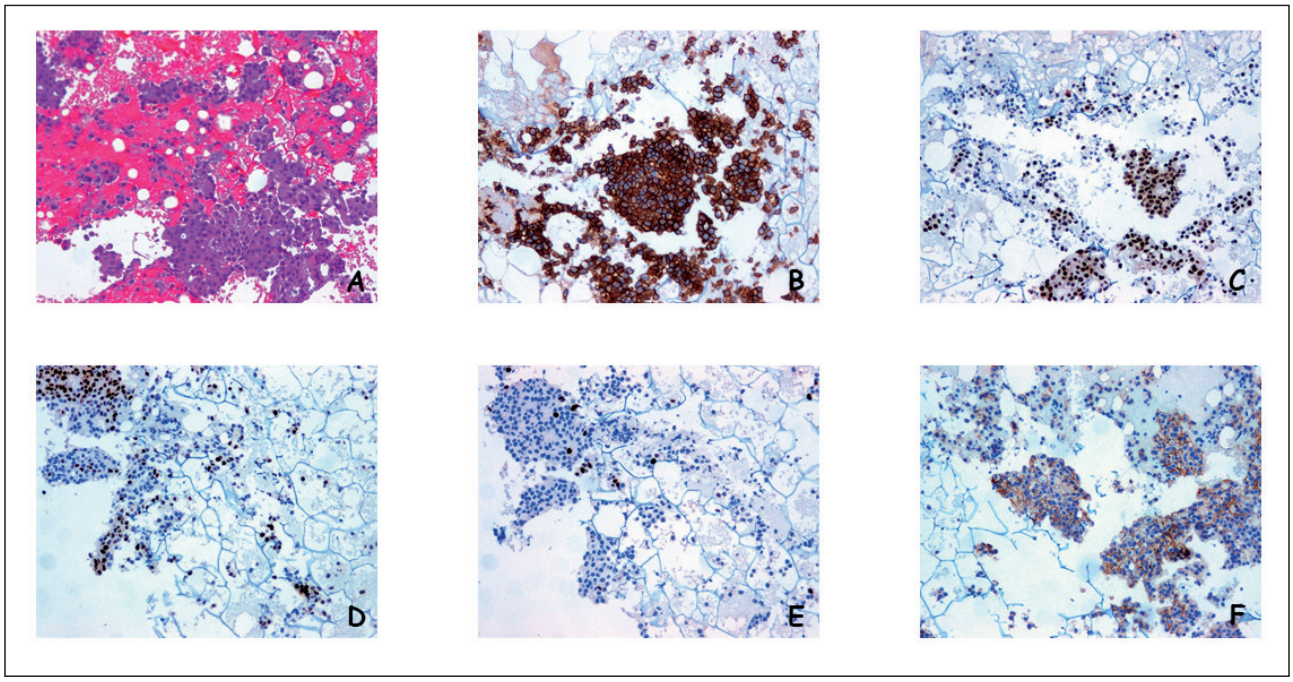

Figure 1. *Histological and immunohistochemical pattern of the material collected by Cytomatrix from a fine needle aspirate of a lump of the breast.* ***a***. *Histological analysis allowed the final diagnosis of ductal carcinoma. To note, the material stained with eosin is the remaining of the synthetic matrix (H&E, original magnification X10).* ***b***. *Immunohistochemical analysis showed high expression of E-Cadherin (ABC, original magnification X10).* ***c***. *The cancer displayed high expression of Estrogen receptor (ABC, original magnification X10).* ***d***. *Expression pattern of Progesterone receptor (ABC, original magnification X10).* ***e***. *Expression pattern of the proliferation marker ki67 in cancer cells (ABC, original magnification X10).* ***f***. *High expression of HER2-neu on the cytoplasmic membrane of cancer cells (ABC, original magnification X10).*

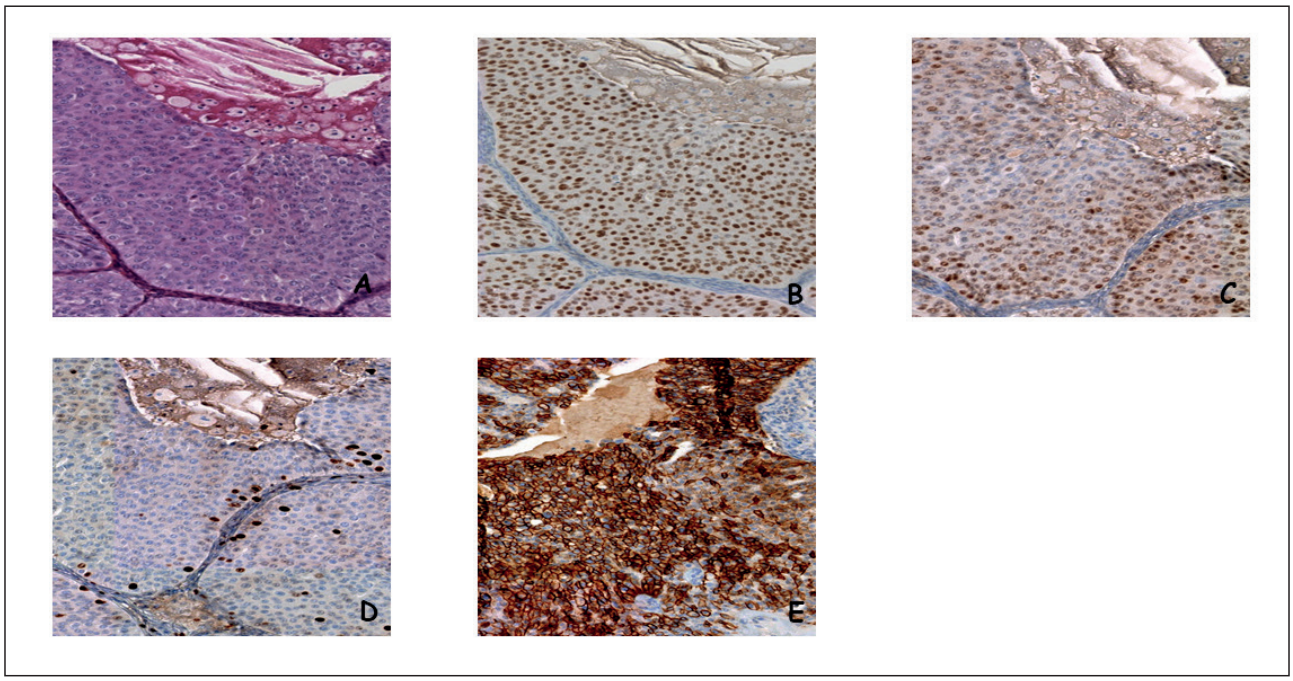

Figure 2. *The histological and immunohistochemical pattern of the same carcinoma presented in figure 1 are overlapping with the data produced with Cytomatrix.* ***a***. *Histological analysis confirmed the diagnosis of ductal carcinoma (H&E, original magnification X10).* ***b***. *The cancer displayed high expression of Estrogen receptor (ABC, original magnification X10).* ***c***. *Expression pattern of Progesterone receptor (ABC, original magnification X10).* ***d***. *Expression pattern of the proliferation marker ki67 in cancer cells (ABC, original magnification X10).* ***e***. *High expression of HER2-neu on the cytoplasmic membrane of cancer cells (ABC, original magnification X10).*

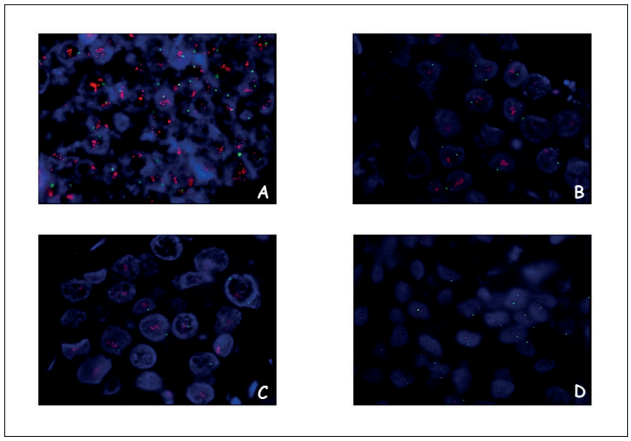

Figure 3. *FISH analysis for HER2-neu of the material collected by CytoMatrix from a fine needle aspirate of two ductal carcinomas showing 2/3+ HER2 immunohistochemical protein expression.* ***a****. In this breast cancer specimen, the signal ratio of HER2 ≥ 2 was regarded as HER2-neu gene amplification.* ***b****. In this breast cancer specimen, the signal ratio of HER2 ≥ 2 was regarded as HER2-neu gene amplification.* ***c****. In this breast cancer specimen, the signal ratio of HER2 ≥ 2 was regarded as HER2-neu gene amplification.* ***d****. In this breast cancer specimen, the signal ratio of HER2 < 2 was regarded as no HER2-neu gene amplification.*

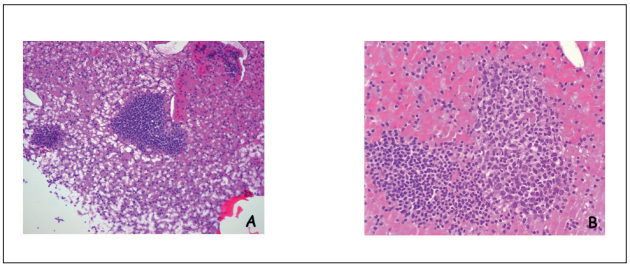

Figure 4. *Histological aspect of the material collected by CytoMatrix from a fine needle aspirate of a neoformation of the parotid gland.* ***a****. Histological analysis allowed the final diagnosis of Whartin's tumour, based on the recognition of the two epithelial and lymphoid tissue components (H&E, original magnification X10).* ***b****. Higher magnification better showing the two different population of cells characteristic of Warthin's tumour (H&E, original magnification X20).*

ETHICS

Fundings

There were no institutional or private fundings for this article.

Conflict of interests

The authors have declared no conflict of interests.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Code availability

N/A

Authors' contribution

MB and AB performed the histopathological analyses, analyzed the data and wrote the manuscript, SM and CLC performed the fine-needle aspirates, CC and AS performed the histopathological and immunohistochemical procedures, MS performed the FISH procedures, EPS contributed in the interpretation of the data.

Ethical approval

The study protocol was approved in accordance with the ethical standards established in the Declaration of Helsinki of 1946 (World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013 Nov 27;310(20):2191-4. Doi: 10.1001/jama.2013.281053).

Consent to participate

Written informed consent was obtained from all the subjects before the collection of the fine needle samples with CytoMatrix.

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ORIGINAL ARTICLE

MALIGNANT MESOTHELIOMA IN THE ITALIAN REGION EMILIA-ROMAGNA: INCIDENCE AND ASBESTOS EXPOSURE UPDATE TO 2020

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ABSTRACT

Malignant mesothelioma (MM) is a rare disease of great interest to the scientific community and to public health due to its high lethality and to its association with asbestos exposure. The aim of this study, is to describe the incidence of MM in the period 1996-2020 with the related exposure to asbestos. The data was collected by the Regional Mesothelioma Registry (ReM) which represents one of the 21 CORs (Regional Operational Centers) in Italy. The study includes all cases of MM with a *certain* or a *probable* diagnosis (with microscopic confirmation) and *possible* cases (without microscopic confirmation). For each case, information on sex, date of birth, tumor site, morphology, date of diagnosis, follow-up and province of residence was retrieved. We collected information on previous occupational and non-occupational exposure to asbestos, by type of sector and activity. The exposure information is collected through the ReNaM (National Mesothelioma Registry) analytical questionnaire, administered to the patient or his closest relatives. The data collection is conducted by a regional survey network. Were registered, between 1996 and 2020, 3013 cases of MM classified as *certain* (85.2%), *probable* (5.4%) and *possible* cases (9.4%). The greatest number of cases are recorded for the pleura (2,763) and for the peritoneum (224) in the province of Bologna, Reggio Emilia, Parma and Modena. The most affected age groups are 65-74 and 75 +. Concerning exposure, 70.3% was defined *professional*, 6.0% *familiar*, 2.3% *environmental* and 1.3% *extra professional*.

As regards the provinces of residence, Bologna holds the primacy for cases of *professional* and *environmental* exposure, Reggio Emilia for *familiar* and Parma for *extra-professional* exposure. Most of the exposure to asbestos is recorded in the construction and in the railway sector, mainly in males.

KEY WORDS

Mesothelioma; incidence; exposure; asbestos.

IMPACT STATEMENT

This paper provides a 25-year overview of the incidence of malignant mesothelioma in Emilia-Romagna, a rare and highly interesting disease associated with asbestos exposure.

INTRODUCTION

Malignant mesothelioma (MM) is a rare tumor of great scientific interest owing to its well-documented correlation with the *occupational* and/or *environmental* exposure to asbestos and the increased incidence recorded in recent years in Italy and in many other industrialized countries (1-7).

In our country, asbestos was definitively banned in April 1994 (see Law 257/92); nevertheless, the long latency time between the beginning of the exposure and the onset of the disease, the lengthening of life and the improvement of diagnostic techniques have led to an increase in the incidence of MM in recent years (8-9). MM remains a deadly cancer with a very poor prognosis, with a median of approximately 10 months from diagnosis (5, 9).

In Italy, the standardized incidence rates per 100,000, recorded in 2013, are equal to 4.2 for male and 1.2 for female, whereas in individuals who were exposed to asbestos the incidence is 100-1,000 times higher. The onset generally occurs after more than 40 years of exposure to asbestos, with a median of 48 ± 11.4 years (8). This pathology can also arise following modest and limited exposure to asbestos: cases have been described in workers exposed to presumably low doses and in relatives of exposed persons who took care, in a domestic environment, of the cleaning of contaminated work clothes. Cases arising from environmental exposure in residents in areas adjacent to industrial settlements where the presence or use of asbestos have also been documented (9). The ReM (Mesothelioma Registry), active since 01/01/1996, is a cancer registry specifically dedicated to the study of the incidence and etiology of MM. The objectives of the ReM, which also performs the functions of COR (Regional Operational Centers) Emilia-Romagna, are the detection of all cases of MM and the acquisition of information for a correct diagnostic definition and standardized attribution of professional or extra-work exposure to asbestos.

The aim of this study, is to report the incidence of MM in the period 1996-2020 with the related exposure to asbestos.

MATERIALS AND METHODS

All cases of MM are detected, with pleural, pericardial, peritoneal, tunica vaginalis testis localization, arising from 1 January 1996 in subjects residing in the Emilia Romagna region at the time of diagnosis. For each registered case, in addition to the reports of the pathological investigations performed, the medical records of significant hospitalizations, carried out in public and private, regional or extra-regional health institutes, are acquired. The information on exposure, both professional and non-working, is collected through the ReNaM analytical questionnaire, administered to the patient or to his closest relatives, by the panel of occupational doctors of the Public Health Departments, members of the regional survey network. Registration for diagnostic definition and exposure attribution follow the standardized rules of the ReNaM (10). The data collection is conducted by a dedicated regional survey network that integrates all public and private Institutes and Pathological Anatomy Services operating on the regional territory, the hospital departments where patients with MM electively converge and all the Departments of Territorial Public Health. The detection network tends to acquire in *real time* the reports of new cases just diagnosed, for the early collection of the information on anamnestic exposure required directly from the patient. To verify the completeness and accuracy of incident cases, link is made with the data period-

	N. CASES	DEFINITION
MM certain	2,566	Histology presents with characteristic morphological picture; characteristic/ suggestive/absent immuno-histochemistry + diagnostic confirmation by imaging/clinical diagnosis of discharge.
MM probable	162	Histology presents with dubious morphological picture or cytology with characteristic picture + diagnostic confirmation by imaging/clinical diagnosis of discharge.
MM possible	285	Absent histology/cytology, indicative clinical and radiological data + diagnosis of MM CC discharge.
		DCO with wording "mesothelioma"
TOTAL	**3,013**	

Table I. *Case Distribution by diagnostic definition, cases from 1996 to 2020 (updated to 12/31/2020).*

YEAR	N. CASES				TOTAL
	SITE				
	PLEURA	PERITONEUM	PERICARDIUM	TESTIS	
1996	63	8	-	2	73
1997	70	7	3	-	80
1998	77	4	1	1	83
1999	67	6	-	-	73
2000	76	9	-	1	86
2001	88	6	-	2	96
2002	98	15	-	1	114
2003	97	6	1	1	105
2004	110	8	2	-	120
2005	107	10	-	2	119
2006	100	7	-	-	107
2007	101	14	-	-	115
2008	122	9	-	1	132
2009	111	11	-	-	122
2010	117	12	1	-	130
2011	144	10	-	1	155
2012	142	10	1	2	155
2013	147	5	-	1	153
2014	122	10	-	1	133
2015	141	10	-	-	151
2016	150	10	-	-	160
2017	146	11	-	1	158
2018	138	14	-	-	152
2019	132	8	-	-	140
2020*	97	4	-	-	101
TOTAL 1996-2020	**2,763**	**224**	**9**	**17**	**3,013**

Table II. *Distribution of cases by site and year of diagnosis.*

* *The incidence is not complete.*

ically acquired from the regional computerized archives (Mortality and Hospital Discharge Records) and information exchanges with the regional population cancer registers and the COR network.

RESULTS

In Emilia Romagna 3013 MM were registered between 1996 and 2020: the distribution by type of diagnostic definition is shown in **table I**. Most of the cases (2,566) were classified as *certain* (85.2%), 162 *probable* (5.4%) and 285 (9.4%) *possible* cases and 6 cases with only the death certificate (**table I**). The distributions by year of diagnosis and localization is shown in **table II**. The cases go from less than 100 per year in the first six years of registration to an average of about 110 in 2003-2007, 130 in 2008-2010 and then reaching around 150 starting from 2011.

In the period 1996-2020 the greatest number of cases are recorded for the pleura (2,763) and for the peritoneum (224), on average 10 cases per year. Tumours in the testis are rare (17), about 1 per year. Those of the pericardium are rare.

Table 3 shows the distribution by year and residence. The provinces in which the majority of cases are recorded are: Bologna (660), followed by Reggio Emilia (463), Parma (355) and Modena (329).

An overview of the distribution of MM in the Emilia-Romagna region referring to the period 2014-2018 (the most recent complete five-year period) is shown in **figure 1**. Compared with the national data for 2013 (males 4.2 and females 1.2), Emilia Romagna has a slightly lower rate in males (3.9) and higher in females (1.4). Compared with the regional data of Emilia Romagna for the period 2014-2018 (3.8 in males and 1.1 in females), it is the province of Reggio Emilia that shows the highest rates in males (5.7) and females (2.3). Following, in males, the provinces of Piacenza (4.9) and Ravenna (4.6); while in the females Piacenza (1.4), Ferrara (1.3) and Parma (1.2). As for age (**table IV**), there is a strong gradient for pleural MM in both sexes. Most of the cases are concentrated in the age of 65 +. For the peritoneum, most cases, regardless of age, are recorded at the age of 65 +; while there are no differences for testis and pericardium.

The incidence trend (**figure 2**) shows a slight increase in males but not females.

Of the 3,013 cases of MM, information on exposure to asbestos was collected in 2,605 cases, 169 not defined and 239 not classifiable. Overall, considering all those exposed to asbestos, in our region it was found that 80% of respondents were exposed (87% males and 60% females) (**figure 3**).

The distribution of type of exposure is shown in **table V**: in 70.3% it was defined *professional*, 6% *familiar*, 2.3% *environmental* and 1.3% *extra professional*. Occupational exposure is much higher in males

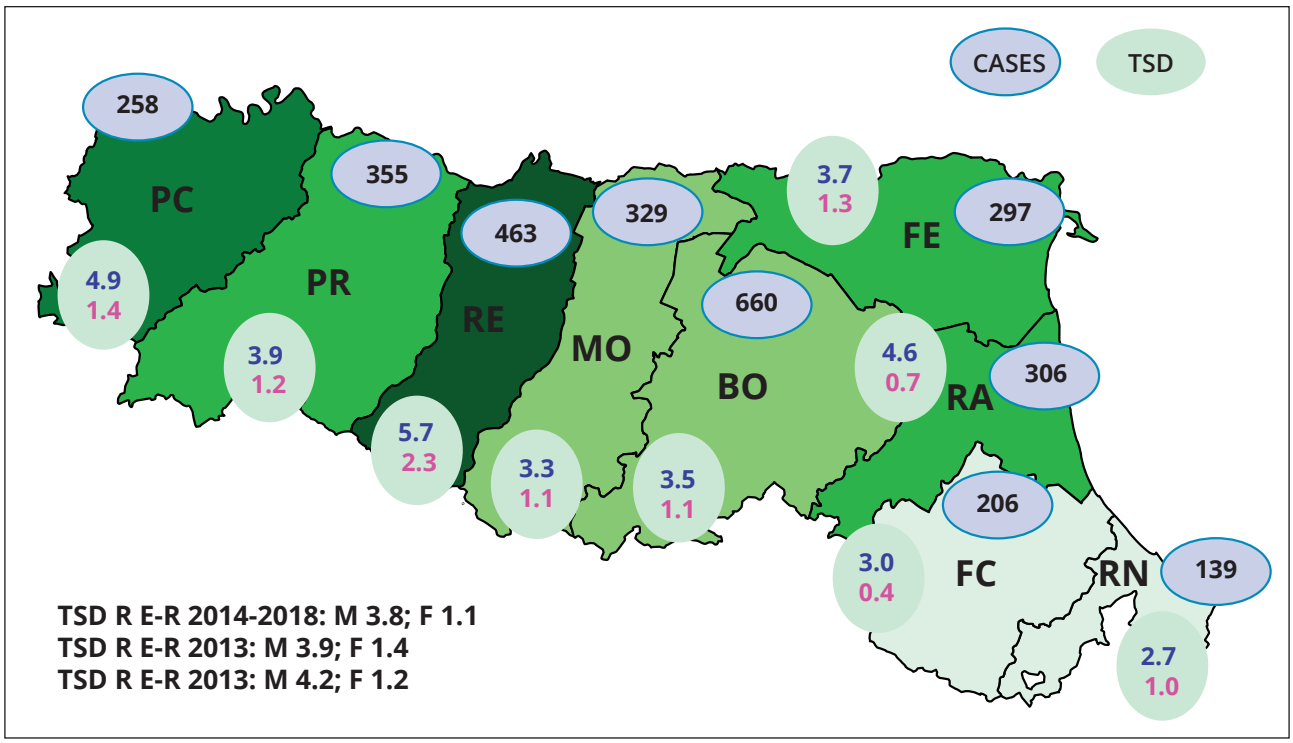

Figure 1. *ReM cases 1995-2020. DIstribution of cases and TSD by province of residence.*

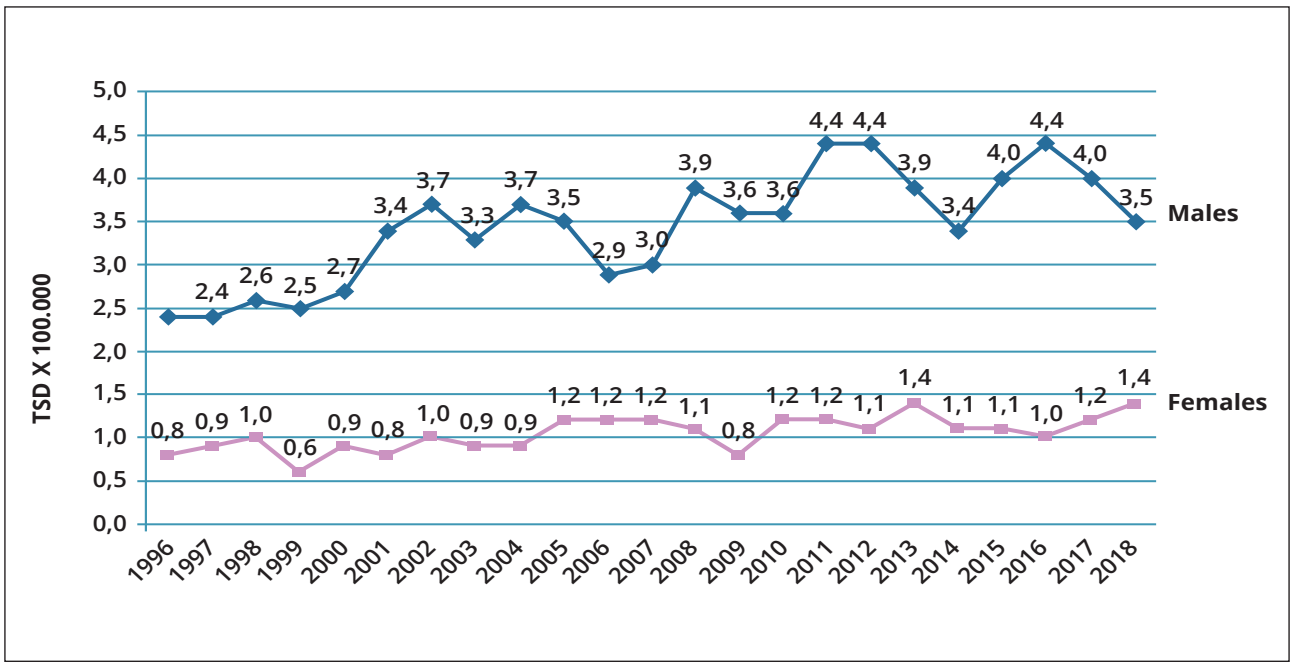

Figure 2. *Standardized incidence rates on the Italian population x 100,000. Years 1996-2018.*

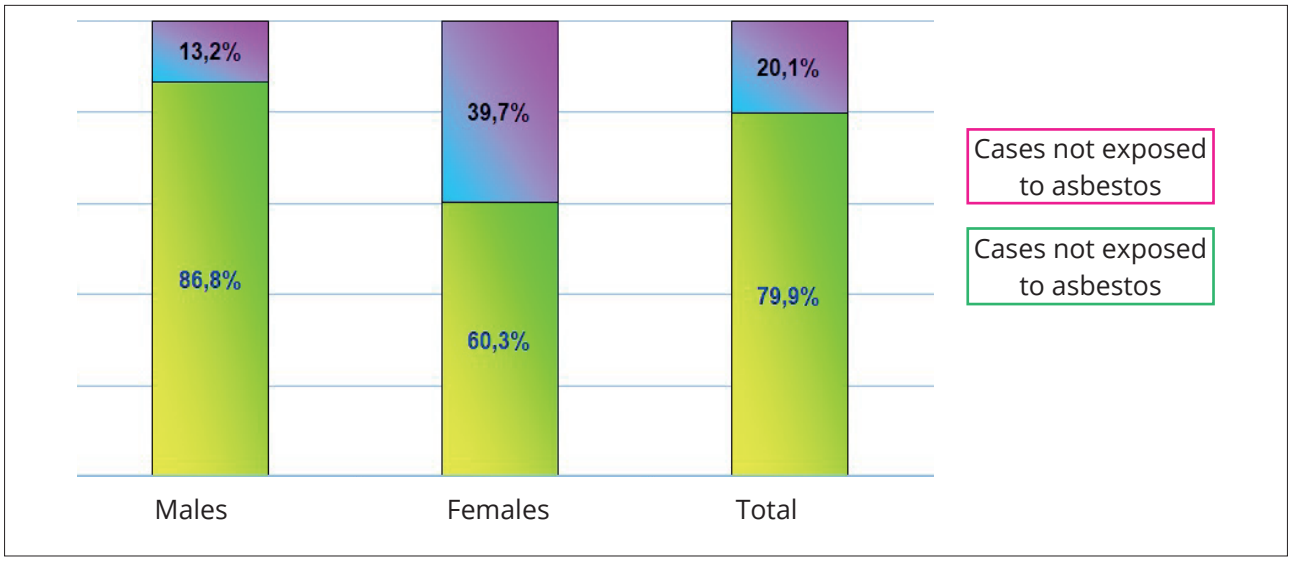

Figure 3. *Distribution by exposure to asbestos by gender in the Emilia-Romagna region.*

(83%) while the family exposure is higher in females (20.6%). As regards the provinces of residence, Bologna holds the primacy for cases of *professional* (400) and *environmental* (19) exposure, Reggio Emilia for *familiar* (38) and Parma for *extra-professional* exposure (7) (**table VI**). **Table VII** shows the working sector involved: most of the exposure to asbestos is recorded in the construction sector and in the railway sector, mainly in males. Women, in general, are less affected, but most of the cases are recorded in the sectors of sugar refineries and in production of cement.

DISCUSSION

Malignant Mesothelioma confirms its characteristics of rare tumor with an increase in the incidence in both genders, recorded up to 1996 and with the first signs of a steady trend in the following years. It should be noted that the ReM data relating to 2019 and 2020, which show a significant decrease, of -8 % and - 34% respectively, compared with the incidence rates recorded in 2018, are probably due to the detection deficit caused by the current viral

YEAR	N. CASES									
	PC	PR	RE	MO	BO	FE	RA	FC	RN	R E-R
1996	5	12	13	8	18	7	7	1	2	73
1997	9	9	10	3	24	7	5	7	6	80
1998	8	8	13	11	20	7	7	6	3	83
1999	7	6	10	6	14	9	7	8	6	73
2000	7	10	10	8	17	13	8	11	2	86
2001	9	13	12	8	22	17	5	5	5	96
2002	8	7	16	12	37	13	11	8	2	114
2003	11	7	16	11	24	10	12	7	7	105
2004	8	16	18	10	24	10	16	12	6	120
2005	13	24	16	12	22	13	9	7	3	119
2006	15	15	10	10	29	10	11	5	2	107
2007	4	19	22	15	28	6	13	6	2	115
2008	9	14	13	20	23	18	13	12	10	132
2009	7	16	10	13	26	16	19	10	5	122
2010	12	16	19	11	26	9	16	14	7	130
2011	14	14	22	22	31	15	18	10	9	155
2012	15	21	29	13	34	16	12	7	8	155
2013	15	16	25	11	35	18	18	11	4	153
2014	12	16	25	19	28	8	16	5	4	133
2015	12	13	20	20	33	23	14	8	8	151
2016	11	17	27	22	33	12	11	13	14	160
2017	17	13	27	20	35	14	15	11	6	158
2018	12	14	36	17	28	11	17	7	10	152
2019	13	22	20	15	24	11	19	10	6	140
2020	5	17	25	11	25	4	7	5	2	101
TOTAL	**258**	**355**	**463**	**329**	**660**	**297**	**306**	**206**	**139**	**3,013**

Table III. *Distribution of cases by province of residence and year of diagnosis.*

AGE	MALE				FEMALE			TOTAL
	N. CASES				N. CASES			
	Pleura	Peritoneum	Pericardium	Testis	Pleura	Peritoneum	Pericardium	
< 45	18	9	-	4	11	5	-	47
45-54	88	10	-	3	36	10	1	148
55-64	336	22	-	2	94	21	2	477
65-74	683	44	4	3	224	29	1	988
75+	904	43	1	5	369	31	-	1,353
TOTAL	**2,029**	**128**	**5**	**17**	**734**	**96**	**4**	**3,013**

Table IV. *Distribution of cases by sex, site and age at diagnosis.*

TYPE OF EXPOSURE	MALE		FEMALE		TOTAL	
	N. CASES	%	N. CASES	%	N. CASES	%
Professional	1,607	83.5	225	33.1	1,832	70.3
Familiar	15	0.8	140	20.6	155	6.0
Environmental	29	1.5	30	4.4	59	2.3
Extra Professional	20	1.0	15	2.2	35	1.3
Improbable	63	3.3	87	12.8	150	5.8
Unknown	191	9.9	183	26.9	374	14.3
TOTAL OF DEFINED CASES	**1,925**	**100.0**	**680**	**100.0**	**2,605**	**100.0**

Table V. *Distribution of malignant mesotheliomas by type of exposure and sex.*

TYPE OF EXPOSURE	N. CASES									
	PC	PR	RE	MO	BO	FE	RA	FC	RN	R E-R
Professional	147	219	314	163	400	177	215	119	78	1,832
Familiar	11	20	38	7	32	19	8	9	11	155
Environmental	4	13	7	5	19	4	3	4	-	59
Extra-professional	5	7	1	4	6	2	4	4	2	35
Improbable	12	20	8	15	41	14	24	8	8	150
Uknown	40	48	26	40	93	33	40	34	20	374
To be defined	6	10	60	42	33	4	6	7	1	169
Not classifiable	33	18	9	53	36	44	6	21	19	239
TOTAL	**258**	**355**	**463**	**329**	**660**	**297**	**306**	**206**	**139**	**3,013**

Table VI. *Distribution of cases by type of exposure and province of residence.*

pandemic, which makes it necessary to adopt ad hoc in-depth studies currently in progress at the Registry with the involvement of the ReNaM CORs. In view of its almost total lethality, however, this disease still assumes social relevance with an impact greater than that of fatal injuries. The INAIL fatal injuries data reported in 2015-2019 continue to demonstrate a lower occurrence, 610 *vs* 761, compared to the incidence of MM, recorded by the ReM in the same period (see INAIL Annual Report, June 2020).

Since the disease is almost always associated with exposures, even modest ones, to asbestos, each new case must be considered a "sentinel event" of previous exposures and carefully evaluated (7, 10-20). Based on these considerations, the primary objective of the ReM is certainly the completeness of the data and the accuracy of the information collected. These aims seem to have been achieved thanks to the capillary regional detection network which also allows a good recording of MM with extra pleural localization. The diagnostic quality can be considered of a good level: 90.5% of cases are accompanied by cyto-histological confirmation owing to the widespread practice in the regional health services of performing biopsy with minimally invasive techniques, which allow examining also elderly patients and/or patients with reduced "performance status".

The involvement of Prevention Services is certainly important because it guarantees a correct anamnestic reconstruction of exposure and the drafting of good quality certifications for INAIL to guarantee the patient and his family members the recognition of the privileged public protection provided for technopathies. In all our cases, these are 249 subjects out of 2,081 with ascertained exposure to asbestos (12.0%), for which it is not possible, under current legislation, to access privileged forms of protection for damage from work.

The extension of the asbestos victims' fund to people suffering from MM due to exposure to "non-professional" asbestos tends to overcome this situation with the provision of an indemnity, albeit *una tantum*; this was introduced on an experimental basis for the three-year period 2015-2017 by the established law 2015 (see L 190/2014 and DIM 04/09/2015), validated for the following

PRODUCTION SECTOR	MALE		FEMALE		TOTAL	
	N. CASES	%	N. CASES	%	N. CASES	%
Constructions	269	16.7	1	0.4	270	14.7
Constructions/repair railway rolling stocks	186	11.6	3	1.3	189	10.3
Engineering industry	151	9.4	12	5.4	163	8.9
Sugar refineries/other food industries	112	7.0	37	16.4	149	8.1
Production of cement/asbestos products	93	5.8	32	14.2	125	6.8
Production of chemical/plastic material	98	6.1	6	2.7	104	5.7
Building completion works	80	5.0	1	0.4	81	4.4
Glass/ceramic/rubber manufacturing	56	3.5	20	8.9	76	4.2
Transportation	72	4.5	3	1.3	75	4.1
Production/repair vehicles (no trains and ships)	65	4.0	3	1.3	68	3.7
Manufacturing/processing of metallic products	60	3.7	3	1.3	63	3.5
Textile industry	35	2.2	15	6.7	50	2.7
Trade	37	2.3	8	3.6	45	2.5
Production electricity, gas, water	39	2.4	-	-	39	2.1
Social services/recreational activities/healthcare	19	1.2	17	7.6	36	2.0
National defense	35	2.2	1	0.5	36	2.0
Agriculture/animal breeding	23	1.4	12	5.4	35	1.9
Metallurgical industry	27	1.7	4	1.8	31	1.7
Other manufacturing industries	27	1.7	3	1.3	30	1.6
Other	123	7.6	44	19.6	167	9.1
TOTAL	**1,607**	**100.0**	**225**	**100.0**	**1,832**	**100.0**

Table VII. *Distribution of occupational exposure to asbestos by main sector of economic activity and sex.*

three-year period 2018-2020 and increased pursuant to art. 11 quinquies, Law 8/2020.

Significantly, the MM recorded in Emilia-Romagna show a high proportion of cases with extra pleural site: the pleura / extra pleural ratio recorded by the ReM was equal to 11.1:1, compared to 13.4:1 and 13,3:1 recorded by CORs in Italy (8, 11) and to some international reports (12, 14) which probably underestimate the data of MM with extra pleural site. Certainly, the articulation of the ReM detection network favours the exhaustiveness of the information collection both from the clinical departments, pulmonology and thoracic surgery mainly, where the MM with pleural site electively flow, and from those where cases with extra pleural site are treated: general surgery, gynaecology, cardiac surgery, urology and andrology. On the other hand, research carried out in the ISPESL/ReNaM field had highlighted some difficulties, especially in some CORs, in the systematic detection of MM with an extra pleural site and had identified possible ways to implement this detection (11).

As regards the age at diagnosis, the average was 71.9 ± 10.7 years; it is noteworthy that 77.7% of subjects were ³ 65 years of age at the time of diagnosis compared to 72.0% recorded in Italy (8). The data could be correlated to a greater tendency, in our region, to perform biopsy sampling even in older subjects, thanks to the good diffusion of minimally invasive practices (*e.g.* video-thoracoscopy) compared to more aggressive traditional methods. The regional annual incidence rates per 100,000, standardized for the 2000 Italian population, show an increasing trend. The years with a higher incidence were 2011, 2012 and 2016 for male (4.4) and 2013 and 2018 for female (1.4) (**figure 2**). The 2014-2018 regional average rates (3.8 M and 1.1 W) are lower than those recorded by the ReNaM in 2013 (4.2 M and 1.2 W). The 2014-2018 TIS show data that cannot be readily interpreted for Piacenza and Ferrara, whereas for Reggio Emilia they are mainly correlated to the significant spread in the past of companies engaged in the production of asbestos-cement products and the construction/repair

of railway rolling stock. In particular, the high value for female is certainly due to the use, peculiar in this province, of female engagement in the manufacturing of "special pieces" in cement/asbestos.
The analysis of exposure to asbestos for the 2,605 cases already investigated, highlighted exposure in 79.9% of cases, whereas for the remaining 20.1% no information was found concerning exposure to asbestos, which defines the class "unlikely/unknown" asbestos exposure. This result, rather than indicating a real absence of previous exposures, even remote and episodic, is likely to be ascribed to the difficulty of recording exhaustive anamnestic, professional or extra-professional exhibition information, concerning situations that could have occurred even a few decades earlier the onset of the disease. These difficulties, more relevant for the female gender, are also linked to the reduced median survival of the MM which does not always allow to detect good quality information from the patient voice.
In most of the subjects exposed to asbestos, the origin of the exposure was traced to professional activities (88.0%), whereas exposure due to cohabitation with professionally exposed subjects or to extra-work activities occurred in 9.1% of cases. Also, among the subjects exposed to asbestos in our Region, the rate of those who developed MM because "they lived near production sites that worked or used asbestos (or materials containing asbestos)" or they have frequented environments with the presence of asbestos for non-professional reasons", the so-called environmental exposure to asbestos (see LL.GG. ReNAM) (10), is equal to 2.9%. This fraction is lower than that recorded by ReNaM in Italy, which is equal to 4.4%, and then that recorded in some Italian municipalities, subject in the past to significant environmental contamination by asbestos.
In the Emilia-Romagna Region, the production sectors most involved in the onset of MM were: building constructions (subjects distributed evenly throughout the region); construction/repair of railway rolling stock (cases mostly resident in the provinces of Bologna and Reggio Emilia); metalworking industry, sugar refineries/other food industries (118 of 149 cases, residing in the provinces of BO, FE, RA, PR, FC), production of asbestos cement products (98 of the 125 cases residing in the province of RE). National ReNaM data, on the other hand, indicate among the most involved sectors, in addition to construction (15.5%) and the engineering industry (8.6%), the textile industry (6.4) and shipbuilding (6.1%).

CONCLUSIONS

The study shows that MM is a rare but still highly lethal pathology. The early diagnosis of the neoplasm, although not able to influence the prognosis, allows to obtain more precise information directly from the patient on previous exposure to asbestos and to ensure, in due cases, the right compensation to those who have paid too high a price during his job duties.

ACKNOWLEDGMENTS

The collection, archiving and definition of malignant MM cases incident throughout the Emilia-Romagna Region was possible, with an acceptable cost/benefit ratio, only through the effective collaboration and careful development of the regional detection network. which has over 140 formally designated Referents1, including: pathologist specialists, hygienists and occupational doctors from the Departments of Public Health, pulmonologists, general surgeons, gynaecologists, urologists, oncologists, but also internists and cardiologists that the good collaboration established can guarantee an increasingly adequate knowledge of this fearful pathology.

ETHICS

Fundings

There were no institutional or private fundings for this article.

Conflict of interests

The authors have declared no conflict of interests.

Availability of data and material

All the data supporting the findings of this study are available within the article and can be shared just before a reasonable request to the corresponding author.

Authors' contribution

LM: conceptualization, investigation, writing-original draft, visualization, supervision; CS: investigation, methodology; IB: writing - review & wediting, supervision. AR: conceptualization, supervision. All authors have read and agreed to the published version of the manuscript.

Ethical approval

N/A

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ORIGINAL ARTICLE

THYROID CANCER IN SARDINIAN PEDIATRIC PATIENTS: REPORT OF 63 CASES AND A REVIEW OF THE LITERATURE

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ABSTRACT

Thyroid cancer is considered uncommon in pediatrics, yet it is the most common endocrine malignancy among them. The aim of this study was to analyze pediatric thyroid carcinomas diagnosed in Sardinian children and adolescents in order to find a possible association with autoimmune diseases.
We studied 63 consecutive 10-20-years-old patients who underwent surgery for thyroid cancer between January 2001 and April 2020 in our hospital.
No evidence of risk factors including external radiation was found. All cases were follicular-derived neoplasms: 45 PTCs (72%), 9 FTCs (14%), 2 well differentiated carcinomas not otherwise specified (3%), 2 poorly differentiated carcinomas (3%), 5 cases of encapsulated PTC-FV were re-diagnosed as NIFTP (8%), according to the last WHO classification. Autoimmune thyroid diseases were detected in the 29 PTCs (64% of PTCs). BRAF V600E mutation was found in 21 PTCs (47% of PTCs). Our study shows that thyroid cancer in Sardinian children and adolescents is characterized by peculiar features: our cohort is composed only by Follicular-derived differentiated thyroid cancer without medullary carcinomas; PTC seems to be more frequent and strongly associated with autoimmune thyroid diseases in our population. Those evidences, together with the absence of any exposure to radiation in our patients, support the possibility that autoimmune diseases became an important event to be considered also in the evolution of pediatric thyroid carcinogenesis.

KEY-WORDS

Pediatric thyroid carcinomas; BRAF V600E; PTC; autoimmune thyroid diseases.

IMPACT STATEMENT

A twenty-year study with a large number of cases of thyroid cancer in pediatric patients in the particular genetic scenario of Sardinia.

INTRODUCTION

Even if differentiated thyroid cancers have always been considered rare in pediatric population (1, 2), recently an increasing incidence of thyroid cancer in adults as well as in children and adolescents was reported (3). In the pediatric population, the only consolidated risk factor is the exposure to radiation. The scientific interest on radiation-associated risk of thyroid cancer, increased in pediatric population after the Chernobyl and Fukushima nuclear accident, was extended to radiation exposure regarding therapeutic procedures. In fact, youngest children are more sensitive to radiation-induced carcinogenesis, the minimal latent period for thyroid cancer development after exposure is as short as 4 years and is dose dependent (1, 2). The reasons associated with this progressive trend are controversial because the incidence growth of thyroid cancer in children may not be justified only by an increase of radiation risk (4).

Other risk factors are involved in thyroid diseases and, among these, we can distinguish genetic (thyroid disease like autoimmune thyroid disorders) and epigenetic events (iodine nutritional deficiency) (4-7). Even though there is no indication of ethnic or race susceptibility in pediatric thyroid cancer, an increased trend was found in different geographic regions of the United States and some genetic peculiarities associated with this cancer are present in the Sardinian Island population (8, 9). In particular, Sardinians are more sensible to autoimmune diseases, strongly associated with papillary thyroid carcinoma (10-12). The recent years have seen an increasing focus on the genes implicated in carcinogenesis, also for the thyroid tumors (13, 14). The genetic mutations are important for understanding the nature of the tumors and they have an implication in the therapy and in the follow-up. One of the most important gene mutations on thyroid cancers is the BRAF V600E mutation (15). This mutation, thought to mimic phosphorylation of the activation site (15, 16), is important not only for the prognosis, but also for the therapeutic response. In fact, radioactive iodine therapy normally has a good response, but its efficacy is variable in BRAF V600E + cases (17). The mutated BRAF V600E protein in fact causes a resistance to iodine absorption, thus making radioactive iodine ineffective (17, 18). Recently it was proposed a specific targeted therapy for thyroid cancer (a BRAF inhibitor) used for both, pediatric and adult population, according to the adult guidelines, and thyroid cancer is not classified differently in children compared to young adults (2). All together these data underline that pediatric and adult's thyroid cancer tend to have a different evolution (more advanced disease and an excellent overall survival rate in children and adolescents); however, most of the studies have been performed in radiation-exposed pediatric thyroid carcinoma and there is no clear explanation for these differences. Therefore, there is a strong need for studies in pediatric population and, for this reason, the aim of this study is to analyze 63 Sardinian pediatric clinical cases of thyroid cancer.

PATIENTS AND METHODS

This retrospective study was approved by the ethics committee of the University Hospital of Cagliari. We examined 63 consecutive cases. All thyroid samples were formalin-fixed and paraffin-embedded. 3-micron thick paraffin sections were stained with Hematoxylin and Eosin for histology. All cases have been reviewed by two experienced pathologists (MLL and AS) and histopathological assessment was performed and re-diagnosed according to the fourth edition of the WHO classification (22). BRAF mutational status was determined using the Diatech Pharmacogenetics piro-sequencing system (Diatech Pharmacogenetics, www/diatechpharmacogenetics.com) performed on formalin-fixed, paraffin-embedded tumor tissues. Neoplastic and non-neoplastic (surrounding) formalin-fixed tissues were microdissected from hematoxy-

lin-stained thyroid tissue and genomic DNA was extracted using the MagCore Automated Nucleic Acid Purification system (RBC. Bioscience Corp., www/rbcbiocience.com). For Immunohistochemical analysis, 3 µm thick sections were obtained from each paraffin block. All reagents were purchased from Ventana Medical Systems Inc. 1910 E. Innovation Park Drive Tucson, Arizona 85755 USA.

All immunostaining procedures were performed using the UltraView Universal DAB Detection Kit (Ref. 760-5000) on the BenchMark Ultra instrument, according to the manufacturer's instructions.

RESULTS

The vast majority of cases was incidentally diagnosed by ultrasonography in the setting of a follow-up for a preexisting autoimmune thyroid disease or during screening programs in schoolhouses. At clinical examination, few patients presented a painless or tender thyroid nodule.

No risk factor, including exposure to ionizing radiation or ingestion of radioiodine, was present in any patient's history. No family history of thyroid cancer was reported in our patient's clinical information.

Distant metastases were never detected. At histology all cases were follicular-derived neoplasms (**table I**). In 58 cases a diagnosis of malignancy was made; 5 cases, already diagnosed as encapsulated follicular variants of papillary thyroid carcinoma, were re-classified as NIFTP (8%), according to the new classification of the WHO (19). The malignant cases included 45 papillary thyroid carcinomas (PTCs) (72%), 9 follicular thyroid carcinomas (FTCs) (14%), 2 well differentiated carcinomas not otherwise specified (3%) and 2 poorly differentiated carcinomas with focal areas of differentiated PTC (3%). PTCs were predominantly observed in females (F/M = 36/9), with a median age of 16.8 years (range 10-20 y); 10 cases were multifocal (22% of PTCs), 1 presented intravascular invasion (2% of PTCs) and 25 showed lymph node metastases (55.5% of PTCs). The extrathyroid invasion was present in 17 PTCs (38% of PTCs) and 29 were associated with autoimmune thyroid diseases (64% of PTCs).

About the 9 cases of FTCs, 7 were in females and 2 in males, with a median age of 17.7 years, ranging from 13 to 20 years at presentation. In 5 patients, intravascular invasion was detected (55% of FTCs), but none presented with lymph node metastases. 2 FTCs presented extrathyroid invasion (22% of FTCs) and 4 were associated with autoimmune thyroid diseases (44% of FTCs). The 5 patients with NIFTP were all females, with a median age of 17 years old, ranging from 12 up to 20 years old at presentation. None of them were multifocal, nor did they present intravascular invasion or lymph node metastases or extrathyroid invasion, as indeed it must be for the diagnosis of NIFTP. One was associated with autoimmune thyroid diseases.

The two cases of well differentiated carcinomas not otherwise specified (WDC-NOS) were found in a 15 and a 19-years-old patients, (median age 17 y), respectively a male and a female. 1 of them presented intravascular invasion (50% of WDCs-NOS). None of them were multifocal, nor did they show lymph node metastases or extrathyroid invasion or association with autoimmune thyroid diseases. The two patients with a diagnosis of poorly differentiated carcinoma

HISTOTYPE	N. (% PER COLUMN)	F/M	MEDIAN AGE (RANGE)	MULTIFOCAL (% PER ROW)	IVI (% PER ROW)	LN METASTASES (% PER ROW)	EXTRATHYROID INVASION (% PER ROW)	ASSOCIATED AITD (% PER ROW)
PTC	45 (72%)	36/9	16.8 (10-20 y)	10 (22%)	1 (2%)	25 (55,5%)	17 (38%)	29 (64%)
FTC	9 (14%)	7/2	17.7 (13-20 y)	0 (0%)	5 (55%)	0 (0%)	2 (22%)	4 (44%)
NIFTP	5 (8%)	5/0	17.0 (12-20 y)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)
WDC-NOS	2 (3%)	1/1	17.0 (15-19 y)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
PDC	2 (3%)	2/0	17.5 (17-18 y)	0 (0%)	2 (100%)	1 (50%)	2 (100%)	2 (100%)

Table I. *Cases.*

PTC: Papillary Thyroid Carcinoma; FTC: Follicular Thyroid Carcinoma; NIFTP: NonInvasive Follicular Thyroid neoplasm with Papillary like nuclear features; WDC-NOS: Well Differentiated Carcinoma – Not Otherwise Specified; PDC: Poorly Differentiated Carcinoma; IVI: IntraVascular Invasion; AITD: AutoImmune Thyroid Disease.

(PDC) were both females, one 17- and the other 18 years old (median age 17.5 y). In both cases we found intravascular invasion (100% of PDCs) and extra-thyroidal extension (100% of PDCs). In one patient lymph nodal metastases were present (50% of PDCs). Both patients had autoimmune thyroid diseases (100% of PDCs). Regarding the subtypes of PTCs (**table II**), 17 showed features of the Classical Variant (PTC-CV) (38% of PTCs), 11 were Follicular Variant (PTC-FV) (24% of PTCs), 8 were diagnosed as Tall Cell variant (PTC-TCV) (18% of PTCs), 6 as Diffuse Sclerosis variant (PTC-DSV) (13.5% of PTCs), 2 were diagnosed as Solid Variant (PTC-SV) (4.5% of PTCs) and 1 as Cribriform variant (PTC-CrV) (2% of PTCs). The distribution of BRAF V600E mutation in papillary thyroid carcinomas (**table III**) was found in 21 out of 45 cases (47%), with a median age of 16.3 years old (range 11-20 years). 7 cases were multifocal (33.3%), 1 case showed intravascular invasion (5%), lymph-nodes metastases were present in 13 cases (62%) and extrathyroid extension in 9 cases (42%). The BRAF V600E mutation was associated with autoimmune thyroid diseases in 15 out of 21 cases (71%). BRAF V600E negative PTCs patients had median age 17.4 years old (range 10-20 years), association with autoimmune thyroid disease was found in 14 out of 24 cases (58%), extra thyroid extension in 8 out of 24 cases (33%) and lymph-node metastases in 12 out of 24 cases (50%). No BRAF V600E mutation was found in any cases of FTCs, well differentiated carcinomas not otherwise specified, poorly differentiated carcinomas and NIFTPs.

DISCUSSION

The trend of the incidence rates for sporadic PTC in childhood and adolescence shows a constant growth (3). The prevalent risk factor for thyroid cancer are the ionizing radiations but other genetic, environmental and lifestyle factors are emerging (20). In fact, an increased proportion of mutations less associated to radiation exposure (BRAF and RAS point mutations) was described in different studies (21-24).

VARIANTS	N. (% PER COLUMN)	F/M	MEDIAN AGE (RANGE)	MULTIFOCAL (% PER ROW)	IVI (% PER ROW)	LN METASTASES (% PER ROW)	EXTRATHYROID INVASION (% PER ROW)	ASSOCIATED AITD (% PER ROW)
CV	17 (38%)	15/2	17.2 (10-20y)	6 (35%)	0 (0%)	7 (41%)	5 (29%)	13 (76%)
FV	11 (24%)	9/2	18.4 (16-20y)	1 (9%)	0 (0%)	3 (27%)	2 (18%)	3 (27%)
TCV	8 (18%)	5/3	15.6 (11-19y)	3 (37.5%)	1 (12,5%)	8 (100%)	4 (50%)	5 (62,5%)
DSV	6 (13.5%)	4/2	16.0 (14-19y)	0 (0%)	0 (0%)	6 (100%)	4 (66,6%)	6 (100%)
SV	2 (4.5%)	2/0	14.0 (13-15y)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	1 (50%)
CrV	1 (2%)	1/0	15.0 (15y)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)

Table II. *PTC Variants.*
CV: Classic Variant; FV: Follicular Variant; TCV: Tall Cells Variant; DSV: Diffuse Sclerosing Variant; SV: Solid Variant; CrV: Cribriform Variant; IVI: IntraVascular Invasion; AITD: AutoImmune Thyroid Disease.

	N. (% PER COLUMN)	F/M	MEDIAN AGE (RANGE)	MULTIFOCAL (% PER ROW)	IVI (% PER ROW)	LN METASTASES (% PER ROW)	EXTRATHYROID INVASION (% PER ROW)	ASSOCIATED AITD (% PER ROW)
BRAF V600E mutated	21 (47%)	16/5	16.3 (11-20y)	7 (33.3%)	1 (5%)	13 (62%)	9 (43%)	15 (71%)
BRAF V600E not mutated	24 (53%)	20/4	17.4 (10-20y)	3 (12.5%)	0 (0%)	12 (50%)	8 (33%)	14 (58%)

Table III. *BRAF Mutation in PTCs.*
PTC: Papillary Thyroid Carcinoma.

In any cases, more multicenter studies are necessary to better understand the relevance of different risk factors thyroid carcinogenesis (25).

This study made in Sardinia, a Mediterranea island without any evidence of radiation contamination, in 63 clinical cases without head and neck radiation exposure, represent one of the firsts example of a possible association between pediatric thyroid cancer and autoimmune thyroiditis. This association was more evident in PTC samples (64% of the cases) but seems to be present also in the 4 out of 9 FTC cases examined (44%). The presence of autoimmune thyroiditis was already described in adults thyroid cancers, in PTC and even if in reduced frequency also in FTC (26-28), in the general population or in areas with an high prevalence of Hashimoto's disease (11-13, 29-31).

These thyroid cancers have been described as less aggressive and with a better prognosis and more frequent in younger patient. On the contrary, our pediatric PTC samples with autoimmune diseases showed, as all pediatric thyroid tumors, a more aggressive pattern (55,5% with LF metastasis and 38% of exstrathyroid invasion), confirming that relevant differences between pediatric and adult thyroid tumor may exist.

In our study, thyroid carcinoma affected more girls than boys, with a 4-fold predominance, in line with more recent studies (2, 3, 32, 33) supporting previous hypotheses on a relevant role played by hormonal determinants in the pathogenesis of thyroid carcinoma (2, 34). The median age at diagnosis, in our cohort, was 17 years (ranging from 10 up to 20) different of that observed in others studies (13.5-14.6-14.7 years) suggesting a possible elderly clinical manifestation in our population (3, 32, 33).

The relationship between chronic autoimmune thyroiditis and thyroid carcinogenesis are still controversial but seems that they could share the same molecular pathogenesis and therefore, these inflammatory events could be considered as preneoplastic lesions (35-37).

Some studies (38) estimated that the BRAF V600E mutation has a lower incidence in thyroid pediatric cancers than in adults, while other studies reported a higher incidence (38-41). In line with the latter, in our cohort, BRAF V600E mutation was frequently detected (47% of PTCs). Even if Hardee *et al*. specify that the BRAF V600E mutation is not associated with a more aggressive clinical course in the pediatric people (41), our and other studies indicated a more aggressive behavior, such as described in adults. Therefore, the relevance of the BRAF V600E mutation seems to be relevant also for the diagnosis of the PTC in the pediatric patients, in particular because is indicative for specific therapy strategies.

PTC was the most common histological subtype diagnosed in our children and adolescents, accounting for 70% of the total. No case of anaplastic/undifferentiated carcinoma was found in our series, confirming the rarity of this entity in young people. In our population, follicular derived differentiated carcinomas represent 100% of thyroid cancers originating in children and adolescents. This finding contrasts with previous reports, indicating medullary thyroid carcinoma appearing in about 10% of patients affected by thyroid cancer in childhood, mostly correlated with MEN2B syndrome (42).

CONCLUSIONS

This study represents one of the first example of pediatric thyroid carcinoma made in a population genetically predisposed to autoimmune diseases. The high percentage of clinical cases associated with autoimmune thyroiditis clearly indicate the opportunity of a screening campaign in these pediatric patients in order to prevent a possible insurgence of this type of cancer and to obtain more diagnostic and therapeutic recommendations. Finally, this and other studies highlights the need to establish a more personalized approach in these clinical cases.

ETHICS

Fundings

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Conflict of interests

The authors have declared no conflict of interests.

Availability of data and material

All the data supporting the findings of this study are available within the article and can be shared just before a reasonable request to the corresponding author.

Authors' contribution

All the authors contributed equally to conception, data collection, analysis and writing of this paper.

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COMMENTARY

A SERIOUS CHALLENGE IN FIGHTING THE COVID-19 PANDEMIC: SARS-COV-2 VACCINES IN ONCOLOGIC PATIENTS

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In 2020, the world started to face the rapid spread of a new coronavirus, SARS-CoV-2, that causes the severe respiratory syndrome called COVID-19. COVID-19 soon developed as a pandemic that, in just over a year and a half, has dramatically affected the lives, social behaviors, and economy globally. The COVID-19 pandemic has caused over 4 million deaths around the world, especially in patients with pre-existing medical conditions that make them more vulnerable. In this regard, patients with cancer are at higher risk of being infected by SARS-CoV-2 and develop severe disease. This is due to both their underlying immunosuppression and the cytotoxic regimens that they receive (1, 2). Furthermore, cancer patients are experiencing delays and modifications of their therapeutic plans due to disruption of the hospital workflows and access to care (3). Fortunately, several pharmaceutical companies, partly supported by the governments, rapidly started to develop COVID-19 vaccines. Indeed, vaccines are widely recognized as the main strategy to overcome the present pandemic and are particularly important for cancer patients. Several vaccines have been approved by local regulatory agencies, including mRNABNT162b2 (Pfizer/BioNTech) (4), mRNA1273 (Moderna) (5) ChAdOx1 nCoV-19 (Oxford-AstraZeneca) (6), Ad26.COV2.S (Johnson & Johnson's Janssen) (7), CoronaVac (Sinovac Life Sciences) (8).

Early in 2021, a pivotal study (4) was published reporting that the mRNA vaccine mRNABNT162b2

(Pfizer/BioNTech) is safe and efficient in preventing COVID-19 infection in the adult general population. Two doses of this vaccine at a 21-day interval led to 95% effectiveness in preventing Covid-19 infection. Vaccine efficacy was maintained across subgroups analysis for age, sex, ethnicity, baseline body-mass index, and the presence of coexisting conditions. Moreover, the safety profile of mRNABNT162b2 is characterized by fatigue, headache, and mild pain at the injection site. Given the optimal results achieved in phase III trial, mRNABNT162b2 was approved for clinical use by multiple local regulatory authorities, and vaccination programs began around the world. However, even if 3.9% of the 18,860 vaccinated individuals enrolled in this trial had a history of cancer, no patients receiving active systemic treatment with immunosuppressive or cytotoxic agents were included in this initial trial (4). Thus, the efficacy of mRNABNT162b2 in patients with cancer and in particular, receiving active treatment is unclear.

Of note, according to the guidelines, mRNABNT162b2 vaccination is also approved for cancer patients and it is administered according to the physician and local recommendation. To provide insight into the efficacy and safety of mRNABNT162b2 vaccination in cancer patients, single Institutions recently reported their experience in prospective studies. In particular, two studies (9, 10) evaluated the efficacy of the Pfizer/BioNTech vaccine in 102 and 232 solid cancer patients, respectively. Massarweh et al. compared the rates of anti-spike antibody response to mRNABNT162b2 vaccine in 102 solid cancer patients receiving systemic therapy and compared to 78 healthy controls (Petah Tikva, Israel). Ninety-two percent of the vaccinated cancer patients had detectable SARS-CoV-2 anti-spike IgG antibodies after the second vaccine dose, whereas in the control group, 100% were seropositive. Nevertheless, the authors observed significantly lower IgG titers in patients with cancer compared to healthy controls (1931 [IQR, 509-4386] AU/mL vs 7160 [IQR, 3129-11 241] AU/mL; $P < .001$). The response to vaccination was not associated with specific cancer histology (9). In this study, the authors did not explore T-cell responses to the vaccine. These data suggest that, even if most of the patients were able to achieve a detectable response after a full schedule of vaccination, their antibody titer might not ensure complete protection.

Another single-center study again in Israel (Haifa) (10) evaluated 232 cancer patients who were receiving active treatment compared to 261 age-matched healthy controls. The authors observed that after the first dose of mRNABNT162b2, only 29% of neoplastic patients resulted seropositive compared to 84% of the control group. Similar results were observed also in subgroup analysis according to age lower or older than 60 years. Importantly, after the second dose of the vaccine 86% of tested patients turned seropositive, suggesting that a full vaccine schedule ensures a positive IgG titer even in a high-risk population. Notably, no serious adverse events related to the mRNABNT162b2 inoculation were observed, confirming its safety profile (10).
Finally, Monin et al. reported the experience of three institutions based in London (UK) (11). In this study, the authors compared the efficacy of the mRNABNT162b2 in cancer patients, in terms of the rate of seropositivity for anti-spike IgG antibodies against SARS-CoV-2, and the safety following each vaccine dose in a cohort of patients with a known diagnosis of cancer (n = 151) compared to a healthy control group (n = 54). Compared to the previous studies described, this cohort included also hematological cancer (n = 56) in addition to solid cancer patients (n = 95). All patients received the first dose of mRNABNT162b2 vaccine on day 1. Thereafter, 25 patients with solid cancer and 6 patients with hematological cancer received a second dose on day 21. Sixty-nine patients with solid cancer and 49 patients with hematological cancer received a delayed boost at around 12 weeks. Two patients (one with solid cancer and one with hematological cancer) died during the study period due to COVID-19 infection. Of the 134 individuals evaluated for anti-S IgG titers at 21 days following first dose vaccination, 32 (94%) of 34 healthy controls, 21 (38%) of 56 solid cancer patients, and eight (18%) of 44 hematological cancer patients turned seropositive. Response to vaccination was not associated with specific histology within solid cancer patients, but serological non-responders were enriched in patients receiving steroids at the time of vaccination (11). Remarkably, the rate of seroconversion was dramatically lower in hematological patients, suggesting that patients with impaired immune system will have partial or null immune protection after vaccination.

To address COVID-19 vaccine responses in hematological patients, several groups have retrospec-

tively studied their patients with leukemia, lymphoma, or myeloma receiving different vaccines. Hematological cancers are already known to be associated with lesser seasonal vaccine efficacy due to the disease condition or related to the immunosuppressive regimens administered (12). Indeed, differently than solid cancer patients, liquid cancer patients receiving treatments that specifically target immune cells, including B-cells, T-cells, and myeloid cells. Interestingly, impairment to generate a proper neutralizing response has been described with mRNABNT162b2 used in multiple disease subtypes. In particular patients with B-cell malignancies are commonly treated with anti-CD20 monoclonal antibodies, resulting in prolonged lymphopenia and impairment to generate serological immunity against COVID-19 vaccine (13-20). Of note, CD19- and BCMA- targeted immunotherapies, including bispecific antibodies, CART and antibodies specifically ablate B-cells and plasma cells (21, 22) and could therefore potentially severely affect the response of hematological patients to COVID-19 vaccines. Other drugs used in liquid cancers, especially BTK inhibitors, such as ibrutinib, and venetoclax are associated with failure to respond to COVID-19 vaccine (13, 15-17). Finally, hematological patients undergoing or treated with allogenic hematopoietic transplant are extremely vulnerable to severe COVID-19 infection, given their chronic exposure to immunosuppressive treatment, leading to a poor overall survival (23). Of note, COVID-19 pandemic, and the relative policies adopted by countries to reduce the spread of the virus, impacted the daily practice of the transplantation centers and their networks globally (24).

Overall, it is becoming clear from these pivotal studies that patients with hematological cancers and patients receiving immunosuppressive treatment are at higher risk of developing severe CVID-19 but also responding less to COVID-19 vaccines. These results suggest that all cancer patients should strictly follow social distancing, masking, and hygiene recommendations to avoid infection with COVID-19 (25, 26). Of course, timely vaccination and booster are essential to increase the chances of response to vaccination. In this regard, we suggest when possible to vaccinate patients several weeks before stating their treatments and receive a third dose or booster upon completion and immune reconstitution. A guided strategy involving antibody-titer monitoring could help identify patients at higher risk of infection (low or absent SARS-CoV-2 antibodies) and recommend further actions. Among solid cancer patients, the solely first dose of mRNABNT162b2 vaccination does not seem to be sufficient to generate a proper neutralizing response. However, after the second dose of vaccine, most of the patients became seropositive (9, 10). Thus, among solid cancer patients, it is imperative to respect the full schedule of vaccination in order to achieve proper protection.

In conclusion, the COVID-19 pandemic still represents a tremendous challenge for physicians treating neoplastic patients. The Delta variant of COVID-19 has even worsened this scenario as it can infect vaccinated patients (27). The most effective measures to reduce morbidity in this high-risk population are the same ones that are valid for the general population (social distancing, masking, hygiene) but should also include prompt testing in case of symptoms or exposure, use of the REGEN-COV (the combination of the two neutralizing monoclonal antibodies casirivimab and imdevimab) antibody treatment when indicated (28), vaccination of close family members, and adjustment of treatment schedules and vaccinations to enhance the chances of immune response.

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Authors' contribution

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