Opinion Paper

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CA 19-9: handle with care

Abstract: Since its inception in the mid-1980s of the 20th century testing for carbohydrate antigen 19-9 (CA 19-9) has raised expectation for an earlier diagnosis and accurate monitoring of several malignant diseases. After almost 30 years, the available evidences have confirmed the appropriateness and usefulness of determining CA 19-9 levels as a prognostic indicator and as a reliable tool for monitoring pancreatic and gastrointestinal cancer, but concerns have been raised about its applications in screening, which is actually not recommended, and in the diagnosis of malignancies, due to several interferences that limit the specificity and to the insufficient sensitivity of this marker. In this paper we aimed to review the basic concepts of CA 19-9 testing and its current applications, with a major focus on the most recent evidences dealing with assay interference, methods comparison and monitoring of malignant diseases. The prognostic value and monitoring recommendations for pancreatic, gastric and colorectal cancers are described in depth.

Keywords: CA 19-9; interference; monoclonal antibodies; neoplastic diseases; pancreatic cancer; tumor markers.

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Introduction

The use of monoclonal antibodies for the recognition of tumor-associated antigens, started on a large scale in the 1980s of the last century, has facilitated the study of carbohydrate determinants, since many monoclonal antibodies with apparent specificity for neoplastic diseases react against antigenic determinants of this type [1]. The sialyl-Lewis *a* antigen (Figure 1) is just one of these: N19-9 monoclonal antibody recognizes precisely the carbohydrate antigen (CA) 19-9. It has recently been shown that in addition to this determinant, tied to a single molecule of sialic acid, there is another form, predominantly expressed in non-malignant epithelial cells (disialyl-Lewis *a*) and which is linked to two molecules of sialic acid. This 'normal' molecule functions as a ligand for immunosuppressive receptors and contributes to maintaining immunological homeostasis of the gastrointestinal mucous membranes. In the early stages of carcinogenesis, inhibition of the sialyl-transferase gene causes a partial synthesis for incomplete bond of the second sialic acid residue and the resulting accumulation of the monosialyl Lewis *a* antigen in tumor cells. During the progressive course of the neoplastic disease hypoxia induces the transcription of several genes responsible for glycosilation involved in the synthesis of sialyl-Lewis *a* and the expression of this determinant is then further accelerated in hypoxia-resistant cells with a high degree of malignancy, which become the predominant clones in advanced tumors with high frequency of hematogenous metastases (Figure 2). Although it was characterized almost 30 years ago [2], the carbohydrate antigen sialyl-Lewis *a* (CA 19-9) is still the most commonly used serum tumor marker for the diagnosis of digestive tumors and, in particular, is the 'standard' for serological diagnosis and monitoring of cancer of the pancreas [3, 4]. However, due to the lack of specificity, this marker has limited value in the diagnosis of early forms of pancreatic cancer [4, 5].

Interference with the assays for CA 19-9

The use of CA 19-9 in clinical practice is made difficult by the presence of interfering conditions that can lead to a transient elevation of the levels of this marker. This phenomenon is common to all immunoassays [6, 7] and must therefore always be considered, especially in populations with a low prevalence of the disease [8]. The two situations at the base of the incidental finding of elevations in circulating levels of CA 19-9 in patients with benign disease are: 1) the inflammatory diseases of the digestive tract and liver disease, especially

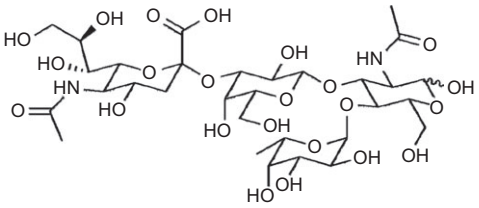

Figure 1 Chemical structure of the sialyl-Lewis *a* determinant.

cholelithiasis; and 2) the false-positivity related to other possible interferences.

The first situation is due to the fact that this molecule is not tumor-specific in the strict sense, hence the determinant is expressed in a small number of normal cells and the expression increases in many non-malignant diseases linked to tissue inflammation and/or to cholestasis. Early after the introduction of the assay in the clinical practice, the presence of false-positives in patients affected by intra- and extra-cholestatic diseases as well as in liver dysfunction have been reported [9–11]. In the first paper Basso et al. investigated the modifications in the serum bilirubin forms, hepatobiliary enzymes, and some glycoproteic substances including CA 19-9 in patients during the course of extrahepatic cholestasis (stage A) and following its clinical resolution (stage B). At stage A, in a number of patients the levels of glycoproteic substances (in particular CA 19-9 and ferritin) were raised, but at stage B they tended to decrease towards the normal range. Extrahepatic cholestasis, in particular, is an important factor in elevating CA 19-9 probably by reducing the hepatic catabolism of this glycoprotein [9]. In two following papers, we evaluated the variations of serum glycoprotein markers in patients with pancreatic that were found to be related to various regional and systemic factors. CA 19-9 and CEA were related mainly to the extent of the neoplasia but the influence of a decreased liver function capacity associated or not with cholestasis and the interrelation with the acute-phase response have been also reported [10, 11]. In addition, an increase of CA 19-9 in patients with lung disease, such as bronchiectasis [adjusted odds ratio (aOR) 2.48; 95% CI 1.22–5.02], bronchiolitis (aOR, 3.93; 95% CI 1.88–8.22), emphysema (aOR, 2.67; 95% CI 1.32–5.40), and interstitial fibrosis (aOR, 10.62; 95% CI 2.03–55.44) has also been described [12]. Therefore, warnings in this sense have been reported later on in the package inserts of all commercial methods. As an example, the package insert of the ARCHITECT 19-9XR assay (Abbott Diagnostics, Wiesbaden, Germany) reports a frequency of values exceeding the given 'normal' threshold of 37 U/L in 4.8% of patients with gallbladder disease (also with very high values, exceeding 1200 U/L), and in 7.2% and 7.4% of subjects with liver cirrhosis or hepatitis, respectively. In a fairly recent Italian study [13] CA 19-9 was found positive with an electrochemiluminescence method in 46% of 56 patients with chronic hepatitis C and in 54% of 60 patients with HCV-related cirrhosis, with levels significantly higher in the latter disease. Recently, an analysis was conducted on 573 patients admitted with suspected pancreatic cancer, that was subsequently diagnosed in 389 cases (62.7%) while 77 patients were not suffering from cancer, 37 had a pancreatic neuroendocrine tumor, 28 a cholangiocarcinoma, four carcinoma of the gallbladder or

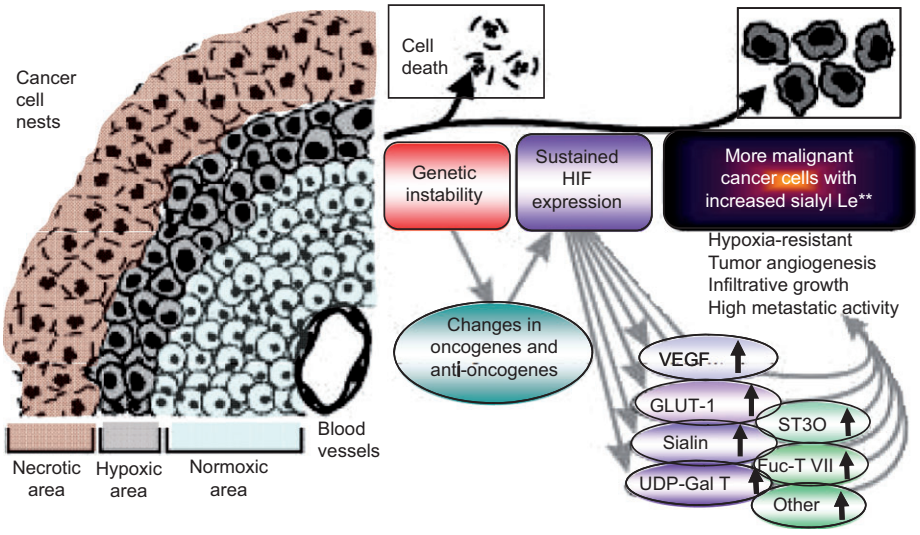

Figure 2 Expression and release of CA 19-9 in cancer progression.
FUC-T-VII, fucosyltransferase VII; GLUT-1, glucose transporter; HIF, hypoxia-inducible factor; Sialin, sialic acid transporter; ST3O, sialyltransferase; UDP-GALT, UDP galactose transporter; VEFG, vascular endothelial growth factor.

ampullary carcinoma and 38 a periampullar cancer [14]. Considering a threshold of 37 U/mL with the Roche Cobas CA 19-9 assay, abnormal levels were found in 27% of non-cancer patients, in 81.5% of pancreatic cancer, in 85.7% of cholangiocarcinomas and in 18.9%–63.6% of other tumors. Significantly higher concentrations ($p<0.0001$) of CA 19-9 were detected in pancreatic cancer and cholangiocarcinoma (median, respectively, of 407 and 345 U/mL) compared to other malignancies, and in pancreatic cancer CA 19-9 levels were lower in surgically treatable forms and in tumors located in the distal part of the pancreas, while higher levels were recoded in patients with liver metastases. Using a threshold of 50 U/mL, or 100 U/mL in patients with jaundice, CA 19-9 was a diagnostic aid for the cancer of the pancreas (sensitivity 77.9%, specificity 95.9%) and specificity varied between 90% and 100% by using different thresholds according to the location of the tumor and the levels of bilirubin.

The most relevant of the demonstrated causes of interference are the presence of rheumatoid factor (RF) and of heterophilic antibodies. Both situations have been reported in the literature: Berth et al. [15] have reported a case of high level positivity for high RF (900 kU/L) associated with a very high positivity (80,000 U/L) with an assay for CA 19-9 (Centaur, Siemens Healthcare) but not with three other assays (ARCHITECT and AxSYM, Abbott, and Vidas, Biomerieux). In the divergent data reported by Liang et al. [16], a patient with a history of biliary polyps and a single result for CA 19-9 of 395 U/L 7 years before showed very different levels of CA 19-9 in 2008 with AxSYM (1,047 U/L) and Elecsys (12 U/L) and was positive for also for RF (122 IU/mL). The authors, however, could exclude that in this case the RF was the basis of the interference, since 18 other samples strongly positive for FR were completely negative for CA 19-9 by the AxSYM assay, and were instead able to attribute experimentally the false-positivity in the presence of heterophilic anti-mouse antibodies. Regarding this last case, a very recent experience [17] has determined the interference from heterophilic antibodies was at the base of 44.4% of the discrepancies observed between two automated assays for CA 19-9: noteworthy, with one of the methods (Roche Cobas) the interference was detected in all discrepant samples analyzed, while with the other (Abbott ARCHITECT) it was found only in six samples out of 15 (40%), but with much higher levels.

The problem of managing clinical results of highly elevated CA 19-9 was perceived from the beginning of 'history' of the determination of this biomarker. For instance, already in 1993 Osswald et al. [18] have reported that, while the frequency of values >500 U/L was 4.6% in a series of 832 measurements, 18% of these values was not related to malignancies, and 97.1% of patients with CA 19-9 elevation in this cohort suffered from gallstone disease.

A different relevance and impact has the retrieval of elevated CA 19-9 levels in healthy subjects, a topic that has been the subject of two recent reports. Ventrucci et al. [19] studied 10 patients with various diseases other than biliary or pancreatic, consistent elevations of CA 19-9 (112–1338 U/L) with an immunoradiometric test and negativity with various imaging techniques and endoscopy. During a follow-up of 2–7 years none of the patients developed any malignancy and CA 19-9 levels have remained persistently high in all cases.

Remarkably similar results have been reported in a much larger cohort in the study of Kim et al. [20], in which the authors enrolled prospectively between 2004 and 2007 a total of 501 subjects (0.8% of all patients tested) with asymptomatic elevation of CA 19-9 above a threshold of 37 U/L with a RIA test. The prospective analysis was subsequently conducted with a follow-up of at least 6 months in 353 cases: a diagnosis of malignancy was made in 10 of these (2.8%), while 97 patients (27.5%) had a benign disease and in 246 (69.7%) reactivity was considered completely unspecific. The authors have proposed an algorithm in which initially abnormal CA 19-9 levels are checked by retesting and, if confirmed, an abdominal CT scan is performed. If no anomalies are detected, CA 19-9 is further checked after 1, 3 and 6 months: a rising trend requires further evaluation (ERCP, MRI or PET), while if the levels are stable or decrease further monitoring of CA 19-9 levels is recommended. It is of note that in 14.7% of cases of non-specific reactivity levels showed no changes throughout the observation period.

The false-positive results for CA 19-9 in populations of patients with benign disease are therefore inevitable, as is well known especially in the case of inflammation of the pancreas, liver or biliary tract, accompanied or not by litiasis that further increases the levels by obstruction of the outflow tract and the consequent accumulation of the carbohydrate antigen in the bloodstream. The only analytical method to determine the malignant nature of these elevations, which often lead to hospitalization and costly and cumbersome procedures, such as diagnostic imaging, is to assess the relationship between sialyl-Lewis *a* and its benign counterpart, disialyl-Lewis *a*, which is not elevated in malignant disease [21]. Recently, Partyka et al. [22] have demonstrated, by the use of 'arrays' with five different antibodies on samples of patients with cancer of the pancreas and with pancreatitis, significant differences between the two groups as well as on single specimens;

some antibodies are highly specific for sialyl-Lewis a, while others identify also a structure related thereto, called sialyl-Lewis c. This finding, subsequently confirmed on a larger cohort, suggests that the use of different antibodies can lead to a better sensitivity in patients with malignant disease without an increase in reactivity in the absence of said disease.

Alternatively, the monitoring of CA 19-9 levels by repeat testing at a time interval between 3 and 12 months can identify possible pathologies in the initial stage (with increased values of CA 19-9 in the second sampling) or, conversely, increases compared to the cut-off with no clinical significance. It shall also be remarked that the knowledge on the biological variability of this biomarker enables the critical difference (RCV) between values to be calculated.

A second issue, the reverse of the previous one, is the dependence of tissue expression, and circulating levels of sialyl-Lewis *a* on the Lewis blood group. The expression is greater in Lewis a+b-, while subjects a-b- have low or no expression and a-b+ individuals have variable expression depending on the level of the fucosyl transferase activity [23]. The a-b- subjects should be entirely negative for CA 19-9 and in about 5%–10% of Caucasians, CA 19-9 is not expressed [3], but it has recently been reported that low or medium (>100 U/mL) levels of CA 19-9 may be found in some patients with this genotype and suffering from advanced pancreatic cancer [24], probably due to the situation of homozygosity for the secretory gene and overproduction of glycan precursors [25]. Obviously, these genetic factors influence the sensitivity of testing for CA 19-9 and not the specificity.

Methods comparison

The radioimmunoassays that were initially used for the measurement of CA 19-9 in the blood and other biological fluids have been progressively replaced by enzyme immunoassays, which are now almost all automated. While this development had an improving effect on the assays imprecision [26, 27], the correlation between the results obtained with different methods progressively worsened: the concentration of CA 19-9 in a given sample, determined with assays from different manufacturers [26–28] or even the same manufacturer but with different tools and technologies [25] can vary quite considerably in some samples. The four studies that have addressed this issue in a systematic manner report substantially similar data and draw strikingly similar conclusions: Passerini et al. [26], in a comparative evaluation of four methods (ARCHITECT, AxSYM, Elecsys, Kryptor), showed that while all assays have good intrinsic performance (CV <10%, no carryover, 100% sensitivity), the same or very similar thresholds of positivity (34 or 37 U/L) and a fair to good correlation coefficient (r between 0.91 – Elecsys vs. ARCHITECT – and 0.98 – ARCHITECT vs. Kryptor), the correlation on higher values was weak, with average differences even exceeding 100% on individual samples. The authors conclude by invoking the principle of keeping the same assay in monitoring patients with malignant disease, and hoping for a better standardization of methods. A similar conclusion was reached also La'ulu et al. [27], who conducted a similar experience on five assays (ARCHITECT, ADVIA Centaur, Unicel DxI 800, Immulite 2000 and Elecsys E170). The authors have shown the same good intrinsic characteristics of the various methods in the face of a correlation variable between 0.85 and 0.98 (the best was between Centaur and ARCHITECT) and a slope of between 1 and 2.06, even in this case with major differences on high values. This 'bias' is in fact proportional and apparently not predictable based on the single evaluation of the correlation between the two analytical methods, as on individual samples either an overestimation or an underestimation of the result may be detected by all methods. It is also evident that the differences cannot be attributed solely to the use of different antibodies, since major discrepancies may be detected also between assays employing the same monoclonals from Centocor: the variables involved in an immunoassay are many (e.g., dilutions, incubation times, reaction kinetics) and all combine to generate a result that, even in the presence of the reference standard (currently not available for CA 19-9) may also vary considerably. Starting from the premise of the lack of interchangeability of the results obtained by different methods, Hotakainen et al. [28] have recently conducted a comparative assessment that was both analytical, on 610 samples from patients with diseases of the gastrointestinal tract, and clinical, on 68 patients with benign diseases and 106 with malignancies (including 30 pancreatic and 43 colorectal cancers). Of the three assays compared in this study (ARCHITECT, Elecsys and Immuno 1), the one that showed a better separation between negative and positive samples was ARCHITECT, with an area under the ROC curve of 0.90, which was significantly greater than those obtained with the other two methods (respectively, 0.78 and 0.76). The authors noted, however, that the best value in discriminating between benign and malignant coincides for all three tests with the value indicated by the respective manufacturers (37 U/L). Apparently, the most recent techniques for CA 19-9 testing will not solve the issue of discordant values: Zur et al. [29] have carried out a purely analytical comparison

of the new luminescent oxygen channeling immunoassay technology on the Dimension Vista 1500 and a classic luminescence assay on the Immulite 2000 XPI and observed lower values by the former method, especially in the low measuring range <100 U/L (r=0.85; slope=0.73). Finally, the most recent study was carried out in Italy by comparing the ARCHITECT and Cobas methods on 500 consecutive routine samples [17]. The results indicate a good qualitative agreement 90.6%, ranging from 79.6% to 100% among different cancer types (lowest for pancreatic cancer), a good correlation (r^2=0.865) and a small number (6, or 1.2%) of highly discordant results, defined as values exceeding 100 U/L with one method and below the threshold with the other. As already mentioned, the interference from heterophilic antibodies was presumably the cause of most of the discrepancies and anyway, apart from this, the authors stressed the need to perform clinical monitoring using the same method. It is worth mentioning that in this study, conducted at a highly specialized center (European Institute of Oncology, Milan) only 13 (2.6%) of the 500 samples were obtained from patients with carcinoma of the pancreas, while 19 (3.8%) and 130 (26.0%) were from patients with gastric or colorectal cancer, which are the other two diseases for which there is a strong indication or recommendation for use of CA 19-9, while the majority of specimens (271, or 54.2%) were obtained from patients with gynecological cancers.

Monitoring of pancreatic cancer

One of the more frequent uses and indeed the most suitable for tumor markers is the surveillance after surgical treatment for a primary carcinoma and/or after chemotherapy [30]. The purpose of this surveillance is the early detection of relapses and/or metastases, and this practice is based on the assumption that this early diagnosis of recurrence or metastases and the subsequent beginning of a new therapy may increase the likelihood of cure or guarantee a better outcome for the patient. However, for most cancers an obvious benefit of this approach is still to be demonstrated [31].

As already mentioned, CA 19-9 is the marker of choice in the management of patients with ductal carcinoma of the pancreas [4], a serious disease for which the survival at 5 years in advanced cases is <5% [32], while studies of the last decade have shown that up to 27% of patients with localized cancer who complete a multimodal therapy survived at least 5 years [33]. Many studies have shown that serial determinations of CA 19-9 can identify recurrences/metastases of pancreatic cancer several months before clinical or radiological evidence of disease (Table 1). Despite the clinical value of this early diagnosis is still uncertain, the European Group on Tumor Markers (EGTM) has recently recommended the use of CA 19-9 in post-surgical follow-up of patients with pancreatic cancer [4], although it expressed caution on the clinical value of starting a new treatment only on the basis of a rise in values of this marker. In accordance with the guidelines of the American Society of Clinical Oncology (ASCO), the measurement of CA 19-9 cannot by itself be considered as a definitive evidence of a recurrence of the disease without being confirmed by imaging and/or a biopsy [34]. However, the exact frequency for measurements of CA 19-9 was not indicated, nor was defined which an increase in CA 19-9 levels may be considered clinically significant.

With regard to chemotherapy, its use in patients not surgically treated is palliative, even if it is only to increase the survival of patients and to improve their quality of life [35]. Assessing the response to systemic therapy in patients with advanced pancreatic cancer using only methods of diagnostic imaging can be difficult because of extensive desmoplasia and surrounding inflammatory components, which can make the objective evaluation unreliable, inaccurate and poorly reproducible. In addition to this, new therapies such as the use of inhibitors of the epidermal growth factor (EGF) have cytostatic rather than cytotoxic effect. These difficulties have encouraged many research groups to use serial measurements of CA 19-9 grouped for assessing response to treatment and/or determining the prognosis in patients with advanced pancreatic cancer treated with systemic therapy. An initial evaluation of 43 consecutive patients with advanced pancreatic cancer treated with gemcitabine [36] has provided encouraging results: patients showing decreased levels of the marker by at least 20% from baseline after 8 weeks of therapy had a better survival (268 days vs. 114: p<0.001) than patients in whom the values remained unchanged or increased, and the decline of CA 19-9 was the independent factor most predictive of response on multivariate analysis. The positive-predictive value of the decline of CA 19-9 was not confirmed in all studies. Stemmler et al. [37] have looked at 77 patients who underwent adjuvant chemotherapy and presented elevated CA 19-9 levels before therapy: all but one of the 14 patients evaluated as 'responders' by CT scan showed a decrease of CA 19-9, but levels fell also in 29 'non-responders' for a positive-predictive value (PPV) of the decrease of 31%; the survival was also significantly higher in patients with a decrease in CA 19-9 (median 295 days vs. 174). In one of the most extensive studies [38], conducted on 76 patients with advanced pancreatic cancer treated

Table 1 CA 19-9 and monitoring of pancreatic cancer.

Setting	Patients (number)	Variation of CA 19-9 levels	Outcome measures and key results	References
Chemotherapy monitoring	43 consecutive patients in advanced stage	Decrease ≥20%	Patients with a decrease of CA 19-9 values from baseline >20% after 8 weeks (n=25) had a longer survival (268 days vs. 110; p<0.001). By multivariate analysis, CA 19-9 was the best independent predictive factor for survival	[34]
Chemotherapy monitoring	77 patients with raised baseline levels of CA 19-9	Decrease ≥50% after 2 months	All the 14 patients evaluated as 'responders' by CT scan but one showed a decrease of CA 19-9 levels. Since levels were decreasing also in 29 'non-responders', the positive-predictive value for decrease was 31%. Survival was significantly higher in patients with decrease (295 days vs. 174)	[35]
Chemotherapy monitoring	46 with advanced/ metastatic cancer	Decrease >20% after 8 weeks	Decrease of CA 19-9 in 13 patients, including the seven responders (2 complete response, 5 partial response) and six non-responders. Longer survival (median: 383 days vs. 242) in patients showing decrease, no one attained normal levels (<20 U/mL)	[42]
Chemotherapy monitoring	Randomized therapeutic trial on 319 patients	Decrease ≥50% after 6 weeks	CA 19-9 evaluated in 175 patients. Mean survival 5.3 months in patients > median level (59 U/mL) at baseline and 10.3 months in patients < median (p<0.0001). Neither the 50% decrease after two cycles of therapy nor the nadir value were associated with longer survival	[37]
Chemotherapy monitoring	47 patients, palliative chemotherapy	Decrease of 25%, 50% and 100%	Weak correlation between the lower CA 19-9 levels after treatment (percentage decrease from baseline) and disease-free survival. No correlation between the kinetics of CA 19-9 during therapy and overall survival	[38]
Diagnosis and monitoring	Review, five papers considered	Decrease >40%–50%	At a theshold of 37 U/mL the overall sensitivity and specificity are 81% and 90%, respectively. Pre- and post-surgical levels are correlated with survival. The employ of CA 19-9 levels in conjunction with imaging techniques is recommended in treatment monitoring. The critical variation of CA 19-9 levels is between 40% and 50%	[4]
Monitoring after surgery	269 patients over 7 years	Not defined	Pre-surgery levels >37 U/mL (38–4,600,600) in 218 patients; 136 (62%) normalized the levels during the follow-up. Lymph node metastasis (p<0.001) and raised levels of CA 19-9 (p<0.0001) independent predictors for limited survival by uni- and multivariate analysis. Raised levels of CA 19-9 after surgery associated with hepatic recurrence and peritoneal metastases	[49]
Monitoring after surgery and chemotherapy	260 patients (88 with measurable CA 19-9 levels pre- and post-surgery)	Not defined	Independent prognostic value for survival after resection (median: 25.6 months vs. 14.8; p=0.0052). No benefit from adjuvant chemotherapy for patients with CA 19-9 >90 U/mL (p=0.719), median survival 26.0 vs. 16.7 months (p=0.011). Levels <37 U/mL after 6 months from surgery independent factor for survival (median: 29.9 months vs. 14.8; p=0.0004). Better prognosis if perioperative values are normal (survival after 5 years: 42%)	[50]
Monitoring of second-line chemotherapy	47 inoperable patients with metastases	Decrease ≥50% for at least 6 weeks	Treatment with gemcitabine+oxaliplatin+5FU. Cumulative survival 15.7 months, survival at 1, 2 and 3 years: 70.2%, 21.3% and 12.8%. Reduction of CA 19-9 levels in 12 patients (26%), associated with progression-free survival and RECIST response, the latter confirmed in 50% of patients with decrease vs. 15% of patients with no decrease	[51]
Monitoring of second-line chemotherapy	206 patients over 8 years	Decrease >20%	Upper normal level 60 U/mL. Patients had been previously treated with gemcitabine. Overall survival: median of 10.3 months in patients with decreasing levels vs. 5.2 months in patients with stable/increasing levels (p=0.008)	[53]

(**Table 1** Continued)

Setting	Patients (number)	Variation of CA 19-9 levels	Outcome measures and key results	References
Diagnosis and monitoring	Review, 14 papers considered	Decrease of 20%–50%	CA 19-9 has a sensitivity and specificity of 79%–81% and 82%–90%, respectively for the diagnosis of pancreatic cancer in symptomatic patients. Patients with normal (<37 U/mL) pre-operative levels (<37 U/mL) have a prolonged median survival (32–36 months) compared to patients with levels >37 U/mL (12–15 months). CA 19-9 levels <100 U/mL associated with likely resectable disease, levels >100 U/mL suggest unresectablity or metastatic disease. Normalization or a decrease in post-operative CA 19-9 serum levels by ≥20%–50% from baseline following surgical resection or chemotherapy associated with prolonged survival	[48]
Monitoring after surgery	154 patients	Not defined	Recurrence at 6 months in 39/73 (53%) patients with values >100 U/mL (best cut-off by ROC analysis) vs. 9/81 (11%) in patients with values <100 U/mL ($p<0.001$). Values >100 U/mL significant predictors of recurrence by multivariate analysis. Median survival 31 months for levels <100 and 16 months for levels >100 ($p<0.001$)	[54]

with fixed dose gemcitabine, CA 19-9 was evaluated as a surrogate marker of clinical outcome, finding a significant correlation between the rate of decline of CA 19-9 both with overall survival and with the onset of treatment failure. Patients with a decrease in levels of at least 25% showed a better outcome than those who did not reach a similar decrease, and the authors therefore concluded that measurements of CA 19-9 should be considered a potential surrogate outcome measure in clinical trials of new therapies for pancreatic cancer. Indeed, opposite conclusions were reached by Hess et al. [39] who analyzed the prognostic value of CA 19-9 levels and of their decrease for response to treatment in patients enrolled in a randomized trial of two different types of chemotherapy (gemcitabine or gemcitabine + capecitabine). No significant correlation was found between the levels of CA 19-9 and response to therapy on 175 patients examined, irrespective of the decline in levels of the marker (20%, 50% or 75%), in particular, it raises concerns that 11 of the 23 patients with progressive disease had shown a decrease of 50% or more of CA 19-9 levels. Furthermore, Klapdor et al. [40], studying 47 patients treated with palliative chemotherapy with a short-term follow-up that required at least monthly determinations of CA 19-9 and CT or MRI every 2 months, have found only a weak correlation between lower levels of CA 19-9 induced by therapy (percentage decrease from baseline) and survival without clinical progression, and no correlation between the kinetics of the marker during therapy and survival.

By contrast, many studies including some of those already mentioned [3, 33, 37, 41–50] indicate that the levels of CA 19-9 before treatment have an independent predictive value for survival. This observation was recently confirmed in the study by Hata et al. [51] who have enrolled over 7 years 269 patients with invasive ductal carcinoma treated with surgical resection, in which the levels of CA 19-9 have been measured before and within 3 months after. The pre-operative levels were above the proposed cut-off of 37 U/mL (range 38–4600) in 218 subjects and returned to normal in 136 of those (62%), while only one of the patients with levels <37 U/mL before surgery showed high levels at the next control. At the univariate and multivariate analysis lymph node metastasis ($p<0.001$) and elevated levels of CA 19-9 ($p<0.0001$) were independent predictors of poor survival, while elevated levels of CA 19-9 after surgery were associated with recurrence in the liver and peritoneal metastases. Humphris et al. [52] have evaluated the correlation between perioperative levels of CA 19-9, the survival and response to adjuvant chemotherapy in a cohort of 260 patients treated by surgical resection for pancreatic cancer. In the subgroup of

patients with measurable values of CA 19-9 pre-surgery, the authors found an independent prognostic value in low levels of the marker after resection (median survival 25.6 vs. 14.8 months, p=0.0052) and before the beginning of adjuvant chemotherapy. In detail, patients with levels of CA 19-9 >90 U/mL did not benefit from adjuvant chemotherapy (p=0.719) compared to those with levels ≤90 U/mL (median: 26.0 vs. 16.7 months, p=0.011). The normal levels (<37 U/mL) after 6 months was an independent favorable prognostic factor (median: 29.9 months vs. 14.8, p=0.0004) and perioperative values identified a group of normal patients with a more favorable prognosis, associated with a 5-year survival of 42%. In the evaluation carried out recently in a most clinically challenging setting of 47 patients with unresectable metastases treated with gemcitabine + oxaliplatin [53], the change in levels of CA 19-9 was considered significant for a decrease by at least 50% from baseline lasting at least 6 weeks [54]. While patients with higher levels of the marker showed a trend for reduced progression-free survival [PFS, hazard ratio (HR) 1.8], 12 patients (26%) had reduced levels of CA 19-9, associated with longer PFS (9.5–11.5 months, HR 0.82 and the decrease was associated with clinical response (odds ratio 5.2, 1.3–14 lc 95%, p=0.02), which was found in 50% of them and only in 15% of patients without reduction of CA 19-9 levels. In another recent experience carried out on 206 patients previously treated with gemcitabine and subjected to different regimens of second-level chemotherapy [55], a variation of 20% in CA 19-9 levels was associated with better survival (10.2 months vs. 5.2, p=0.008), but in this study, the threshold for normal levels was raised to 60 U/mL. An even higher threshold has been employed by Sugiura et al. [56], who assessed the impact of preoperative levels of CA 19-9 on early recurrence and found a significantly higher recurrence rate in patients with initial levels >100 U/L (53% compared to 11% for patients with levels <100 U/L; p<0.001). The definition of 'normal' values of CA 19-9 is not so unique, and is generally based, unlike the latter case, on the cut-off value suggested by the manufacturers of diagnostic tests, which is usually set at the 95th percentile of observed values in a reference population of healthy subjects, or of patients not suffering from oncological diseases. This approach, and a recent evidence on the direct correlation between CA 19-9 values and diabetes, implies that 'abnormal' values may be found also in non-cancer patients, and vice versa, as confirmed by the literature. A very recent review by the European Group on Tumor Markers (EGTM) [4], who examined all recent papers on this issue, indicates that a threshold value of 37 U/mL allows to reach a sensitivity of 81% (very predictable, given the absence or reduced expression of the marker in certain Lewis phenotypes) and a specificity of 90%. The authors also confirm that the pre-and post-surgery levels correlate with survival and recommend the use of CA 19-9 in combination with diagnostic imaging in monitoring, with a clinical variability in the order of 40%–50%. A comprehensive analysis of the potential applications of CA 19-9 in pancreatic cancer diagnosis and monitoring has been recently provided by Ballehaninna and Chamberlain [50]. Table 1 summarizes the data of the literature on the clinical usefulness of CA 19-9 in pancreatic cancer.

Monitoring of gastrointestinal cancers

Indications for use of CA 19-9 in monitoring gastric cancers after surgical resection were expressed by the National Academy of Clinical Biochemistry (NACB) [57], but the evidence in this regard are not univocal and substantially fewer than for pancreatic cancer. One of the main reasons for a lower relevance of CA 19-9 in this pathology is the limited expression of this marker: Passerini et al. [17] have shown that only 30% of patients with gastric cancer had levels higher than 37 U/L, and the mean values were significantly lower than in pancreatic cancer patients (10 vs. 283 U/L). Likewise, analyzing a cohort of 1439 patients who underwent curative gastrectomy for advanced gastric adenocarcinoma, Kwon et al. [58] noticed that only 102 patients (7%) had levels higher than the cut-off value of 37 U/L. From here, the potential clinical utility of assessing several markers in gastric cancer has been prospected. Thus, it is indeed worth remembering the report of Japanese authors [59] which contains data of a national study conducted at 135 centers on 321 patients followed for at least 5 years after surgery. Of these, 120 (34.7%) showed a relapse during the observation period. In the course of monitoring both carcinoembryogenic antigen (CEA) and CA 19-9 were used, the sensitivity for recurrence were, respectively, 65.8% for CEA, 55% for CA 19-9 and 85% for both. Compared to diagnostic imaging, CEA identified recurrences from 12 months before to 5 months after and CA 19-9 from 13 months before to 10 months later. These authors contend that the usefulness of both markers in monitoring, especially for patients with high pre-surgical levels in which an increase associated with the recurrence was detectable in 94% of cases. However, the need to assess carefully the increases of both CEA and CA 19-9 in patients with gastric cancer emerged from the assessment of Kim et al. [60] which, applying as an evaluation

criterion for both markers a change in the levels at least 20%, have detected an increase in values from baseline followed by a decrease in seven of 40 patients (18%) who showed, moreover, a radiological evidence of response to therapy. The median onset and duration of the non-specific increase of the two markers were, respectively, 2.8 and 9.1 weeks. In an effort to improve the specificity of both CA 19-9 and CEA determination in these patients, some authors have also suggested raising the threshold for positivity, indicating an increase of at least 5 ng/mL for CEA and 100 U/mL for CA 19-9 as significant [61].

The correlation between CA 19-9 and gastric malignancy is less than that found in pancreatic cancer, and this has led several research groups to assess the association of several biomarkers both for prognosis and in the monitoring of gastric cancer. In this context there are two case-control studies. In the first one [62] the authors evaluated AFP, CEA and CA 19-9 on 52 patients gastrectomized for gastric CA and in 52 controls: at least one marker was positive in 20 cases (38.5%) and in seven controls (9.6%) and the best predictive value was attributed to AFP at diagnosis and to CA 19-9 at relapse. In addition, CEA positivity was associated to liver involvement and CA 19-9 to peritoneal dissemination. The second study [63] was a retrospective analysis of 9 years of 512 patients who underwent surgical treatment, for 142 of whom (71 with and 71 without recurrence) complete data were available. Of the three markers considered, CA 72-4 appeared to be the more sensitive (35.2%) than CEA and CA 19-9, while the combination of all three brought the sensitivity to 62%. The same was done for the diagnosis of peritoneal metastases, with a range from 33.3% for a single marker to 66.7% for the association of the three. Using a threshold value of twice the reference limit for CEA and CA 19-9, the specificity of these markers increased, respectively, to 98.6% and 94.4%. The authors suggest the combined use of CEA, CA 19-9 and CA 72-4, indicating how the persistence of high values or the increase is strongly suggestive of recurrence. The most recent observation is from Korean authors [64], who evaluated the correlation between the levels of perioperative CEA, CA 19-9 and CA 72-4 and recurrence of gastric cancer in a retrospective analysis of 479 patients with a follow-up period of 5 years. In patients with advanced stage gastric cancer the sensitivity for disease relapse was 100% for CEA, 68.2% for CA 19-9 and 51.3% for CA 72-4, and multivariate analysis showed that an increase in post-operative CEA was an independent prognostic factor in early-stage tumors, whereas in patients in advanced stage independent prognostic factors were age >60 years, stage III and post-surgery increase of CEA and CA 72-4. All three markers have proven so useful in the follow-up of patients with advanced gastric cancer, although CA 19-9 and CA 72-4 showed a low sensitivity and all three showed a high rate (60%–97.2%) of false positives.

Although there are no recommendations ad hoc, it has been suggested for several years [65] and is also quite common [17] to also recommend the use of CA 19-9 in epithelial malignancies of the colon and rectum. In these conditions it is important to have prognostic indexes for selecting the best treatment strategies, and to this end serum tumor markers have also been assessed, mainly in association with each other. Yang et al. [66] evaluated the prognostic value of pre-operative levels of CA 19-9, CEA and CA 125 for 5-year survival without relapse in 103 patients. The initial positivity for CA 19-9, CEA and CA 125 was associated with a higher frequency of recurrences (75.0% vs. 41.0%, 65.6% against 39.4% and 87.5% vs. 44.2%; for all $p<0.05$), and patients with a combined positivity for all three markers had a recurrence rate of 100% and the shorter survival (median of 4 months). By multivariate analysis only stage and status of the association preoperative CEA + CA 125 + CA 19-9 were independent prognostic factors. The clinical usefulness of the association of multiple markers has been highlighted by a recent Italian study [67], whose aim was to evaluate the diagnostic significance of simultaneous measurement of five markers (CA 19-9, CEA, CA 72-4 osteopontin and CYFRA 21-1) in a homogeneous population of 102 selected patients with colorectal carcinoma (CRC) and 99 controls matched by sex and age and with benign colorectal diseases. Ostepontin showed the best sensitivity (45.1%) and CEA the best specificity (90.9%) but the overall accuracy was poor, ranging from 24.9% to 67.2% for CA 19-9 for CEA. The combination of the five markers in any case enables a sensitivity of 74.1% and a specificity of 94.3% to be achieved.

The observations in the monitoring of patients after surgery and/or chemotherapy for colorectal cancer (CRC) are scarce, and generally deal with the use of multiple markers. It is worth mentioning the study of de Haas et al. [68] who evaluated both CEA and CA 19-9 in comparison with the radiological response (TAC) to chemotherapy after complete resection for metastases of CRC, considering a change in levels of at least 20% as significant. Serial determinations of CEA and CA 19-9, respectively, were available for 113 and 68 patients: the patterns of these markers, or biological evolution, was similar to the radiological evidence of response in 94% of cases for CEA and in 91% of cases CA 19-9, and in patients with radiologic progression the correlation with the performance of the serum markers was, respectively, 95% and 64%. It is of note that the progression of CA 19-9, and not the radiological response, was an independent predictor of clinical PFS, and the

authors concluded that the use of tumor markers may be sufficient to evaluate the response to chemotherapy, limiting the need for repeated radiological investigations. Two other very recent studies have evaluated the usefulness of biomarkers in the differential diagnosis and monitoring of CRC: Holdenrieder et al. [69] have studied the levels of CA 19-9, CYFRA 21-1 and CEA in 42 patients with CRC, 45 with benign disease and 51 healthy individuals, whereas the same markers as well as TPA, TPS and M30 antigen were assessed in the monitoring of primary therapy in 15 patients with CRC and correlated with treatment response and survival. The best discrimination between healthy controls and patients with CRC was obtained by combining the results of CYFRA 21-1 and CA 19-9 [area under the ROC curve (AUC): 86.7%], while the combination of CEA and CA 19-9 best discriminated between benign and CRC (AUC=73.9%). In patients with CRC during primary chemotherapy the levels of all markers except CA 19-9 and M30 tended to be higher in patients with poor response and poor prognosis, although the small number of observations does not allow meaningful conclusions to be drawn. By contrast, in a cohort study on 72 patients with high baseline levels of CA 19-9 and/or CEA treated with an oxaliplatin-based chemotherapy regimen with the addition of bevacizumab, a humanized monoclonal antibody directed against the angiogenic vascular endothelial factor, both markers showed a prognostic value [70]. In the discrimination of the progressive disease compared to stable disease/partial response/complete remission the AUC at different concentrations thresholds was 0.83 for CEA and 0.80 for CA 19-9. The elevation of one or both of the markers, here evaluated at a threshold of 28% for CEA and 22% for CA 19-9, was an early signal of progression. At this threshold, CA 19-9 had a sensitivity of 89.7% and a specificity of 59.3%, while the combined use of two markers carried the sensitivity to 84.8% and the specificity to 98.6%. The complementarity of CA 19-9 e CEA in monitoring patients with colorectal cancer has been recently underlined by Lin et al. [71], who analyzed over time a cohort of 385 patients with normal CEA levels and demonstrated a lower rate of disease-free survival at 5 years in patients with high basal levels of CA 19-9 (82% vs. 68%; $p<0.001$), together with a higher frequency of lung metastases.

Table 2 shows an overview of the experiences described above.

Clinical variability of CA 19-9

As shown in the previous sections, a basic unclarified point is to what the extent a decrease/increase of CA 19-9 is clinically significant. The clinical variability of a quantitative parameter is expressed as a function of the analytical variability, i.e., the imprecision of the measuring instrument, and the biological variability, which is often difficult to determine with certainty. There are few systematic studies on the topic to which to refer: Plebani et al. [30] have evaluated four tumor markers, including CA 19-9, with RIA assays of serial samples obtained from healthy subjects and cancer patients: for CA 19-9 an intra-individual variability of 15.9% and an index of individuality of 0.85 were measured, which led to the conclusion that a critical difference of 44.7% should be considered. Vestergaard et al. [72] have studied the biological variability of CA 19-9 and the secretory Lewis genotype in 500 healthy individuals, suggesting the reference ranges for different genotype, since the upper limit of reference was, respectively, 12.4 U/mL and 61.2 U/mL in secretory and non-secretory genotypes. The analytical imprecision was 9.8%, the intra-individual variability 15.5% and the inter-individual variability 102.2%, and based on these values the critical difference should be 51.1%, thus very similar to that indicated in the previous study, suggesting that in the follow-up of patients only a change of levels of at least 40%–50% should be considered as clinically relevant. Similar considerations have been proposed more recently by Erden et al. [73], who studied CA 19-9, CEA and AFP in 49 healthy volunteers, highlighting for CA 19-9 an intra- and inter-individual variability of 27.2% and 64.2%, for a critical difference of 64.7%.

Concluding remarks

The analysis of the available literature data leads to the following conclusions:

- The different methods for the determination of CA 19-9 may provide different results, and it is therefore important that the report contains the indication of the method used and, instead, is wholly inappropriate to consider the results obtained with different methods as interchangeable.
- The harmonization of results should be pursued with existing programs for methods assessment and correction of bias, as was the case with other immunoassays (e.g., insulin and PTH).
- Falsely elevated results due to interference situations are possible with all methods and these assays shall not be used for screening, a situation in which the positive-predictive value of any biomarker is still very poor.

Table 2 CA 19-9 and monitoring of gastrointestinal cancer.

Setting	Patients (number)	Variation of CA 19-9 levels	Outcome measures and key results	References
Monitoring after gastrectomy	321 patients (national study, Japan)	Not defined	Both CA 19-9 and CEA considered. Follow-up of at least 5 years, recurrence in 120 patients (37.4%). Sensitivity for recurrence: 85% for CA 19-9, 65.8% for CEA. Compared to diagnostic imaging, CEA identified recurrences from –12 to + months, CA 19-9 from –13 to +10 months. Both markers are useful especially when pre-surgery levels are high	[57]
Monitoring after gastrectomy	52 with gastric cancer and 52 controls	Not defined	The positivity for AFP, CEA and CA 19-9 were evaluated. At least one marker was positive in 20 cancers and 7 controls. A high predictivity was observed for AFP (for diagnosis) and for CA 19-9 (for recurrence). CEA positivity was associated with liver involvement and CA 19-9 with peritoneal involvement	[60]
Chemotherapy monitoring	40 for CA 19-9 and 51 for CEA	Increase or decrease >20%	The same criteria suggested by other authors (20% increase followed by 20% decrease) were applied. This evenience occurred in 7 patients (18%) with a median time for recurrence of 2.8 vs. 9.1 weeks. However, those patients showed response to treatment by imaging criteria	[58]
Monitoring after gastrectomy	71 cases with recurrence and 71 controls	Not defined	Median follow-up 33 months, markers evaluated at least every 3 months after positivity. Better sensitivity for recurrence for CA 72-4 (35.2%), raised to 62% when combined with CA 19-9 and CEA. The same for peritoneal metastases (from 33.3% to 66.7%). The specificity of CEA and CA 19-9 raised to 98.6% and 94.4% when threshold values were set at twice the conventional values	[61]
Monitoring after gastrectomy	479, retrospective evaluation	Not defined	In advanced stages the sensitivity for recurrence was 100% (CEA), 68.2% (CA 19-9) and 51.3% (CA 72-4). Age >60 years, stage III and post-surgery increase of CEA and CA 72-4 independent prognostic factors by multivariate analysis. The three markers were useful in the follow-up of advanced stages; CA 19-9 and CA 72-4 had a low sensitivity and all three showed a high percentage (from 60% to 97.2%) of false positives	[62]
Monitoring after gastrectomy	102 with advanced cancer and positive baseline values	Normalization	Normalization, defined by levels <37 U/mL after 6 months, achieved in 79 of 102 patients, no association with preoperative levels. By multivariate analysis, pathological lymph node metastasis ($p<0.001$) and CA 19-9 normalization ($p=0.001$) were independent prognostic factors	[56]
Monitoring after hepatic resection and chemotherapy	68 for CA 19-9 and 113 for CEA, patients with metastases	Decrease >20%	Similar behavior of tumor markers and imaging for response in 94% of cases for CEA and 91% for CA 19-9 and for progression in 95% of cases for CEA and 64% for CA 19-9. CA 19-9 progression and radiologic non-response independent predictive factor for PFS. The employ of tumor markers may allow to evaluate the response to chemotherapy, limiting the need for repeated imaging procedures	[66]
Monitoring after chemotherapy	15, evaluation of five other markers	Not defined	Progression in six patients (30%). During primary chemotherapy the levels of all markers, except CA 19-9 and M30, showed a tendency to be more elevated in patients with low response and worse prognosis	[67]
Monitoring after chemotherapy and treatment with monoclonals	56 (CEA was also evaluated on 69 patients)	Decrease >25%	AUC for the discrimination of progressive disease vs. stable disease/partial remission/ complete remission: 0.83 for CEA, 0.80 for CA 19-9. Raise of levels for one or both markers (28% for CEA, 22% for CA 19-9) was an early indicator of progression. At the proposed threshold CA 19-9 had a sensitivity of 89.7% and a specificity of 59.3%; the combined use of both markers decreased slightly the sensitivity (84.8%) but raised the specificity to 98.6%.	[68]

- The threshold value for CA 19-9 depends on the context or the clinical question. While a threshold of 37 U/mL appears best meet the need for discriminating between benign and malignant diseases, for prognostic purposes the issue is less defined and is also determined from the time when the measurement is made (either before or after surgery or therapy). As regards the post-surgical determination, a prognostic information can be provided by levels between 90 and 200 U/mL or higher, but important information is assured by comparison with pre-operative levels, and then by the kinetics of decrease.
- It is adequate, though not recommended, to consider the levels of CA 19-9 in the initial assessment (pre-treatment) of patients with pancreatic cancer or gastric or colorectal cancer as a prognostic factor, but not for the therapeutic choice. It is also useful to determine the levels of CA 19-9 in the same diseases after surgery, for prognostic purposes.
- For the monitoring of patients the same method should ideally be used. If the method is changed, the levels of CA 19-9 should be determined by both assays on two to four serial samples for each patient [4] in order to establish new reference values and an appropriate cut-off.
- It is recommended to monitor levels of CA 19-9 in patients with pancreatic cancer after surgical resection and/or in patients treated with systemic chemotherapy, interpreting the results always in association with diagnostic imaging tools.
- Consensus recommendations on the interval of monitoring pancreatic cancer are not currently available: it is suggested that controls should be made at intervals of 1–3 months, but different intervals may be considered depending on the underlying disease, the clinical stage and the type of therapy.
- The determination of CA 19-9 in monitoring patients with gastric or colorectal cancer can give some helpful information, especially in combination with other markers, such as CEA.
- There is not a clear consensus on the extent of clinically relevant decrease/increase of the marker. The analytical evaluations suggest that the critical difference between sequential values of CA 19-9 should be on the order of 40%–50%, but the extrapolation of these data to patients with oncological diseases is not easy. Most of the available clinical evidences indicate as potentially significant as a variation of 50% of the values during the monitoring of pancreatic cancer, while in gastric and colorectal cancer typically smaller variations are evaluated, which allows the sensitivity to be improved but at the expense of specificity.
- The already mentioned presence of interfering factors, as for all immunoassays, the transient elevations reported after initiation of chemotherapy and the reduced positive-predictive value indicate that the results should be interpreted with caution, and the change in the levels of CA 19-9 alone should not lead to the decision to change the therapeutic strategy.

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