Overview on Gastric Cancer

Chapter 6

Tumor-Associated Neutrophils (TANs) in Gastric Carcinomas: Clinicopathological and Prognostic Implications

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1. Introduction

Gastric cancer, the fifth most common cancer worldwide, and the third leading cause of cancer-specific mortality, has very poor prognosis, with a 5-year survival less than 30% [1-2]. The TNM staging remains the cornerstone in clinical oncology to stratify prognosis and establish therapy for patients with neoplasm [3]. Gastric carcinoma is a heterogeneous neoplasm with respect to anatomic location, epidemiology, genetics, histopathology, and biologic behavior, and, consequently, it has been subjected to many different classifications. With respect to anatomic location, gastric carcinomas may be distinguished as proximal (also known as cardia) and distal (also known as noncardia). This classification correlates with distinct epidemiological risk factors. Obesity, hiatal hernia and reflux gastroesophagitis all are associated with cardia carcinoma, whereas H pylori infection is responsible for 77% of distal carcinoma [4-6]. From a histopathological viewpoint, routine classifications include those proposed by Laurèn [7], WHO [8], and Goseki [9]. The Laurèn classification [7] recognizes two main histological types: intestinal and diffuse, which show correlations with distinct clinical and epidemiological features. Intestinal type adenocarcinoma is mainly found in high risk areas of gastric cancer and is associated with the global decrease in incidence of this tumor. Histologically, intestinal type adenocarcinoma consists of tumor cells showing glandular differentiation with tubular, papillary or tubulo-papillary growth pattern. In diffuse type gastric carcinoma, tumor cells show abnormal loss of glandular differentiation and invade the stroma singly or in small groups. The WHO classification [8] is based on the predominant morphological component of the tumor (usually >50%) and identifies five types of gastric carcinoma: papillary, tubular, mu-

cinous, poorly cohesive (including signet-ring cells and other variants) and mixed carcinomas. Goseki classification [9] combines two tumor features, tubular differentiation and amount of intracytoplasmic mucus, in the following four groups: Group I (good tubular differentiation, poor mucus amount in cytoplasm), Group II (good tubular differentiation, rich mucus amount in cytoplasm), Group III (poor tubular differentiation, poor mucus amount in cytoplasm), Group IV (poor tubular differentiation, rich mucus amount in cytoplasm). Recently, The Cancer Genome Atlas (TCGA) research network suggested a molecular classification of gastric carcinoma in four subtypes based on the presence of Epstein-Barr virus (8.8%), microsatellite instability (21.6%), genomic stability(19.6%) and chromosomal instability(49.6%) [10]. This biomolecular classification offers several advantages, showing particular correlations with anatomic location and/or tumor histologic types. Tumors with chromosomal instability occur more frequently at the gastroesophageal junction and in the cardia, whereas Epstein-Barr positive tumors arise more frequently in the fundus and body. Genomic stable tumors show diffuse histology and are characterized by CDH1 and RHOA mutations. Epstein-Barr virus tumors display PIK3CA mutation as well as JAK2 and PD-L1/2 overexpression. Tumors with chromosomal instability exhibit intestinal morphology, marked aneuploidy, TP53 mutation and focal amplification of receptor tyrosine kinases. Microsatellite unstable tumors show elevated mutation rates, including mutations of genes encoding targetable oncogenic signaling proteins. However, from a prognostic viewpoint none of these 4 subgroups of gastric cancers showed any significant survival differences. Although H pylori is responsible for 77% of distal carcinoma, H pylori status was not evidenced in this molecular classification [11-12]. Therefore, further studies are needed to demonstrate that TCGA gastric cancer classification may have practical implications for improving both therapy and survival in these patients.

Gastric carcinoma heterogeneity is reiterated by the strong variability in the host inflammatory reaction. The WHO classification [8] describes four stromal reactions (desmoplasia/scirrhous reaction, lymphocytic infiltration, stromal eosinophilia, and a granulomatous response), neglecting the role of tumor associated neutrophils (TANs) in gastric carcinomas. Traditionally, neutrophils were considered cell protagonists of the acute phase of inflammation, where they play an important role in the defense against microbial invasion. Recent studies have widened this view showing new functions of neutrophils including the orchestration of innate and adaptive immune reactions [13-15]. In recent decades, increasing attention has been paid to the role of neutrophils in tumor-host reaction, but conflicting conclusions on the prognostic impact of TANs have been reported in literature.

In this work, we summarize the current state on the clinicopathological and prognostic implications of TANs to elucidate this problem. Our experience with TANs in gastric carcinomas is also reported and discussed according to recent data from literature.

2. Neutrophil-To-Lymphocyte Ratio(NLR)

Blood counts, such as the NLR, are being used for diagnostic and prognostic aims in patients with cancer [16-22]. Indeed, NLR has also been used for early detection and as a prognostic marker in gastric cancer [23-25], early diagnosis of ovarian cancer [26-27], and prognosis and survival prediction in colon cancer [28] and hepatocellular carcinoma [29]. Recently, NLRs have also been used in the differential diagnosis between primary breast carcinoma and benign proliferative breast disease [30]. In most studies, a high NLR is associated with adverse overall survival in many human tumors. However, NLR data must be considered with caution, as they are nonspecific parameters, which may be influenced by concurrent conditions such as infections, inflammation, and medications [31]. Many studies did not explicitly check for such concurrent conditions, and this may lead to erroneous interpretations [31]. Furthermore, there are few works regarding NLR values in patients with a low/high neutrophil count in the tumor.

3. N1 and N2 – Polarization or Hyperactivation

Experimental studies suggest that TANs show a bipolar pattern of activation (N1/N2) similar to that observed in macrophages (M1/M2) and T-cell (Th1/Th2) polarization [32-33]. N1 neutrophils exert antitumor activities through tumor cytotoxicity, whereas N2 neutrophils favor tumor growth, invasion and metastasis, e.g. through proteolysis of extracellular matrix components, promotion of angiogenesis and mediation of immunosuppression [34-38]. However, N1/N2 neutrophils have only been shown in murine tumor models and must be confirmed in human tumors [39]. It is also possible that the N1/N2 phenotype reflects only a functional state of neutrophil activation [39-40]. For example, neutrophils isolated from early tumors are more cytotoxic toward tumor cells and release higher levels of NO and H_2O_2, whereas in advanced tumors, neutrophils display low cytotoxic activity and acquire a protumor phenotype [41]. These data suggest that tumor stage plays an important role in modeling neutrophil phenotype and function.Therefore, further studies are needed to clarify whether the different functions of TANs can be attributed to distinct subpopulations, or rather to different grades of neutrophil activation in human tumors.

4. Distribution of TANs and Neutrophil Recruitment in the Tumor Stroma

Distribution of TANs is multifaceted. TANs may be found at the invasive front of tumor (peritumoral location) (**Figure 1a**) or in the center of tumor (**Figure 1b**) [42-46]. Intratumoral TAN distribution shows heterogeneity, being found as a single massive infiltrate in the tumor stroma or as a series of multifocal aggregates scattered throughout the tumor stroma. In some cases numerous neutrophils may be found within the tumor epithelium (**Figure 2**) [47]. It has been pointed out that peritumoral TANs are mainly found at the early stage of tumor development, while intratumoral TANs are seen at later stages [48].

4.1 TANs and tumor necrosis

Mechanisms responsible for neutrophil infiltration in the tumors may be manifold. It is a common experience of pathologists to observe neutrophils in the tumor stroma using light microscopy, and, to date, this phenomenon is interpreted as secondary to tumor necrosis. Consequently, areas of tumor necrosis are not taken into consideration during a TAN count. However, the role of tumor necrosis in the recruitment of TANs is not clear. Innate immune cells may differentially react to necrotic cells in terms of chemotaxis e.g. macrophages may show more rapid and sustained attraction toward necrotic cells rather than dendritic cells and neutrophils[49]. Moreover, it is possible to observe large areas of tumor necrosis without neutrophil infiltration (**Figure 3**).

4.2 Classification of cell death.

A number of factors such as hypoxia, starvation or acidosis can determine tumor tissue necrosis. However, the classic notions of apoptosis and necrosis, based on morphologic criteria, have recently been modified. The latest recommendations of the Nomenclature Committee on Cell Death suggest two mutually exclusive types of cell death: accidental and regulated cell death [50-51]. Accidental cell death is an uncontrollable, instantaneous, passive type of cell death that occurs after severe tissue damage due to extreme environmental conditions (e.g. high temperature, elevated pressure, trauma). Morphologically, it is characterized by cellular swelling and consequent collapse of the plasma membrane thereby causing spillage of intracellular contents and inflammatory reaction [51]. Other morphologic changes include generalized swelling of cytoplasm and organelles, nuclear membrane dilatation, nuclear chromatin condensation into small and irregular patches [52]. By contrast, regulated cell death is an active program of cell death that can be modulated by pharmacological agents or genetic interventions both in physiological and pathological conditions. It occurs during chronic or mild exogenous perturbations of cell microenvironment following failure of compensative mechanisms (e.g. autophagy) [50-51]. The term programmed cell death refers to regulated cell death occurring in physiological conditions (e.g. embryonic development, adult tissue homeostasis). A classic example of programmed cell death is apoptosis, which affects single cells or small clusters of cells [51]. Morphologic characteristics of apoptosis include reduction of cell volume (pyknosis), chromatin condensation, nuclear fragmentation (karyorrhexis), plasma membrane integrity until final steps of the process, formation of apoptotic bodies, phagocytosis of apoptotic bodies by macrophages or by adjacent cells (so-called efferocytosis), with no inflammation in most cases [51].

4.3 Subtypes of regulated cell death

Recent studies have however suggested that regulated cell deaths include several subtypes such as necroptosis, ferroptosis, pyroptosis, parthanatos, and mitochondrial permeability

transition–driven regulated necrosis, but not apoptosis [50]. These types of regulated cell death are not mutually exclusive and are not governed by a single molecular pathway, but are characterized by a complex interplay and cross-talk between them [50]. Moreover, they manifest a partial or total necrotic morphology [51]. To date, necroptosis is the best studied form of regulated cell death and is now recognized as an important drug-sensible contributor to tissue injury in many pathologic conditions including ischemia-reperfusion damage, acute inflammatory reactions, and tumor necrosis [50-53]. It is characterized by cellular swelling, rapid membrane permeabilization and concomitant release of damage-associated molecular patterns (DAMPs) into the extracellular space. According to recent studies, DAMPs determine neutrophil infiltration in the tumor stroma [54]. Moreover, neutrophils stimulated by dying cells exert NO/H_2O_2–based cytotoxicity against residual live cells [50-51]. Ferroptosis is a recently described subtype of regulated cell death due to accumulation of lethal lipid ROS produced through iron-dependent lipid peroxidation [55]. Morphologically, it does not show chromatin condensation, characteristically found in apoptosis, nor loss of plasma membrane integrity, as observed in accidental necrosis [50-51]. Instead, it is characterized by mitochondrial shrinkage and increased mitochondrial membrane density [51,55]. These recent revolutionary modifications in the classification of cell death have disclosed new fields of research. It is not surprising that there are a few updated studies focused on the role of regulated cell death in the recruitment of TANs.

4.4 Other Mechanisms of Neutrophil Recruitment in Tumor Stroma

Several literature data suggest that tumor necrosis is not the sole factor responsible for TANs. Immunohistochemical studies have demonstrated that the type of mucin overexpressed by tumor cells is highly correlated with TANs. For example, TANs are prominent in pancreatic and gastric carcinomas with MUC1 overexpression (**Figure 4**) [56-57] and scarce or absent in mucinous carcinomas that overexpress intestinal mucins MUC2 [58]. These data suggest that tumor histologic type may be correlated with TANs. Accordingly, our previous cancer registry study, undertaken to determine the incidence and clinicopathologic features of neutrophil-rich gastric carcinoma in the Messina province (South Italy), revealed an inverse correlation between TANs and mucinous subtype of gastric cancer, classified according to the WHO classification [57]. Mucinous histologic types are recognized morphologically for the presence of extensive extracellular accumulation of mucins that show a strong MUC2 immunoreactivity [58]. Tumor cells discharge MUC2 into mucin lakes that invade tissues and inhibit the host inflammatory reaction [59]. These data explain the scarce neutrophil infiltration in gastric mucinous carcinomas observed in our study (**Figure 5**). In addition to immunohistochemical studies, biomolecular research disclosed the importance of oncogenic activation in the induction of TANs. CXC chemokines [e.g., interleukin-8 (IL-8)], produced by oncogene mutations in cancer cells, may evoke neutrophil infiltration in tumor stroma [60]. One oncogene that has

been strongly linked to the recruitment of TANs is the Ras oncogene. Ji et al. [60] showed a link between the presence of activated K-ras mutations and macrophage and neutrophil predominant inflammation in both murine and human lung tumors. IL8 has been shown to be a transcriptional target for Ras signaling and expression is required for tumor-associated inflammation and angiogenesis in a human tumor xenograft model [61].

Other experimental studies showed that engineered tumors to release interleukins or chemokines in their microenvironment evoke a massive neutrophil infiltration that, often in collaboration with CD8+ T lymphocytes, leads to the rejection of engineered tumor cells and the establishment of significant immunity against wild-type parental tumors [62]. Taken together, literature data suggest that TANs have manifold recruitment causes including tumor necrosis, MUC1 overexpression, and oncogene activation leading to direct cytokine production by tumor cells.

5. Ultrastructural Studies of TANs in Gastric Carcinomas

Our ultrastructural investigations, performed in gastric carcinomas, reiterated the dual face of TANs. At light microscopy, well differentiated gastric adenocarcinomas show intraglandular migration of neutrophils associated to necrotic phenomena of variable extent ranging from single adenocarcinoma cell death to segmental disruption of the epithelial layer of a gland (**Figures 6a, 6b**) up to glandular and stromal necrosis [63-65]. At electron microscopy, we see a spatial relationship between neutrophil intraglandular migration and regulated adenocarcinoma cell death. In Figure 7, one neutrophil is seen in intimate contact with one or a few severely injured adenocarcinoma cells showing increased electron density, loss of microvilli, marked dilatation of nuclear envelope, small condensed chromatin particles, and progressive mitochondrial and endoplasmic reticulum swelling. These ultrastructural changes are different from those described in apoptosis where there is characteristic chromatin condensation and separation of euchromatin and heterochromatin and formation of apoptotic bodies. They are also different from that described in accidental cell death where there is plasma membrane disruption and cytoplasmic organelle swelling. They manifest partial necrotic ultrastructural features, and therefore are compatible with regulated cell death (**Figure 8**). These ultrastructural observations *in vivo* are similar to that reported in *in vitro* studies where neutrophil cytotoxicity requires physical contact between neutrophils and tumor cells [66]. It remains to be investigated whether regulated cell death is triggered by infiltrating neutrophils or alternatively may be responsible for neutrophil infiltration. This question could be resolved through experimental studies of developing disruptions, as opposed to morphologic static observations of disruptions described above.

6. Emperipolesis and Efferocytosis

Humble, in the 1950s [67], coined the term emperipolesis to describe penetration of lymphocytes in other living cells, both in physiological and pathological settings. During emperipolesis, the migrating cell remains viable and can exit without morphological and physiological abnormalities. Efferocytosis is a term used to describe phagocytosis of apoptotic cells, occurring in embryonic development, organogenesis, tissue repair, atrophy, and inflammation [68-69]. Removal of apoptotic cells is necessary to avoid their disintegration in the tissues via a process known as secondary necrosis which leads to uncontrolled leakage of the dying cells and subsequent chronic inflammatory reaction. Macrophages and dendritic cells are the main cells involved in apoptotic cell removal, including apoptotic neutrophils [68-69]. However, also "neighboring" cells such as epithelial cells, endothelial cells, and fibroblasts, may engulf apoptotic cells [69]. In our previous study, we provided morphologic evidence of apoptotic neutrophil efferocytosis by foveolar epithelial cells in chronic active H. pylori gastritis [70].

7. Entosis and Xeno-Cannibalism

In cytological or histological samples of tumors, pathologists can detect cells with cannibalistic properties. This phenomenon may be suggested by the presence of one or more cytoplasmic vacuoles, possibly containing dying cells, that push the nucleus to the periphery giving it the shape of a crescent moon. Classically, the term cannibalism was used to describe the engulfment of tumor cells by other tumor cells. Recently Overholtzer [71] coined the term entosis to describe a process similar to cannibalism and frequently found in human and experimental tumors, whereby cells become internalized into neighboring cells, forming what are called 'cell-in-cell' structures. In his studies, he demonstrated that internalized cells initially appear healthy and viable [71]. Over a brief period, some internalized cells are able to escape, but most cells die through a form of cell death which is distinct from apoptosis as dying cells are negative for cleaved caspase-3, and do not exhibit condensed or fragmented nuclei. Instead, LAMP1, a lysosomal membrane protein, localizes around dying cells and acidification occurs at the earliest stages of death, suggesting lysosomal involvement [71]. Recent reports have shown tumor cell phagocytosis of normal cells (xeno-cannibalism) such as neutrophils, lymphocytes, and erythrocytes [72-75]. These new observations imply that cannibal tumor cells do not distinguish between normal and sibling neoplastic cells.

In literature, there is some confusion about the terms emperipolesis, entosis, cannibalism and xeno-cannibalism [76-77]. In our opinion, they must be used appropriately remembering the neoplastic or non-neoplastic context in which they occur. The main distinctive characteristics of emperipolesis, efferocytosis, entosis, and xeno-cannibalism are summarized in Tables 1a and 1b. Their light microscopic identification in sections stained with hematoxylin-eosin (H&E) sometimes requires ancillary techniques such as caspase

immunohistochemistry, electron microscopy, and TUNEL assay which is based on enzymatic incorporation of labelled nucleotides at sites where DNA fragmentation has occurred. For example, we recently provided light microscope, immunohistochemical and ultrastructural evidence of neutrophil xeno-cannibalism by tumor cells in high grade gastric carcinomas as well as in micropapillary gastric carcinomas (**Figure 9**) [72,78-79]. At the light microscopic level, intra/interepithelial neutrophils showed apoptotic changes such as pyknotic nuclei and cell shrinkage. TUNEL staining documented apoptotic neutrophils in cytoplasmic vacuoles of tumor cells. Electron microscope, a fundamental tool not only to identify apoptosis, but also to discern inter- or intraepithelial neutrophil localization, confirmed the presence of large heterophagosomal vacuoles containing apoptotic neutrophils. Various phases of apoptotic changes were documented in these neutrophils. Ultrastructural signs of early apoptosis included nuclear chromatin separation into dense and electron lucent areas, rounded nuclear profiles, preservation of cytoplasmic granules, and maintenance of cell membrane integrity. Late apoptotic morphology was characterized by cell shrinkage, tightly packed cytoplasmic granules, and uniform collapsed nucleus (**Figure 10**). Secondary degeneration of apoptotic neutrophils in the phagocytic vacuoles of tumor cells included cellular swelling, electron-lucent cytoplasm, vacuolization and indiscernible cell membrane.

The phenomenon of neutrophil xeno-cannibalism by tumor cells may have a series of pathobiological consequences. It has been suggested that the presence of cannibalized cells may interfere with cell mitosis, leading to non-genetic polyploidy [80]. Phagocytosis of neutrophils by tumor cells may constitute a sort of "feeding" activity. Tumor stroma contains malformed microvasculature that contributes to tumor hypoxia, acidosis, and increased interstitial fluid pressures [81]. Thus, independently of microvasculature, tumor cells cannibalizing neutrophils find nutrients useful for their survival. The phenomenon of neutrophil xeno-cannibalism by tumor cells represents an example of protumor activity of TANs, particularly frequent in high grade gastric carcinomas including micropapillary carcinomas.

8. Prognostic Impact of TANs

The prognostic significance of TANs remains controversial. In our previous study on gastric carcinomas, TANs were morphologically identified by H &E stain and manually counted [82]. The multivariable analysis of possible interaction effects of the clinicopathological factors with TANs revealed that female patients with a moderate or extensive amount of TANs had about a 39% reduction in their risk of mortality, whereas no correlation with outcome of male patients was found [82]. A possible explanation for the interaction between TANs and female patients is that sexual dimorphism exists in the immune response [83]. Both humoral and cell-mediated immunity are more active in females than in males, and steroid gonadal hormones may play an important role in regulating this response [84]. These observations suggest the possibility of an inflammatory (neutrophil) and gender-dependent host natural

cytoxicity in the microenvironment of gastric carcinomas. Subsequently, other studies using immunohistochemical stainings (CD66b, myeloperoxidase and CD15) to identify TANs showed discrepant results. Negative (renal [85], hepatic [45,86], colorectal [87], and gastric carcinomas [88-89]), and positive (colorectal carcinomas [47, 90-94]) correlation with patient prognosis have been described in recent publications. These conflicting results may be due to different methods used in quantifying TANs. In several reviews, shift from morphological to immunohistochemical markers was suggested, including CD15, myeloperoxidase and CD66b [95-96]. Nevertheless, both morphological and immunohistochemical methods have their advantages and disadvantages. Galdiero et al. [93] showed that antibodies against myeloperoxidase were not specific for neutrophils, as also monocytes and immature macrophages stained. CD66b is expressed both on human neutrophils and eosinophils and is recognized as a granulocyte "activation marker" [97]. CD15 is expressed on the surface of leukocytes, mainly in neutrophils, eosinophils, and part of monocytes, and do not reflect the activation status of neutrophils [98]. In addition, CD15 has been demonstrated to be expressed occasionally on tumor cells [93].Therefore, CD15 and CD66b are not specific markers for neutrophils, and light microscope analysis of H&E stained sections remains fundamental to distinguish neutrophils from other inflammatory cells. Given that the different markers used to identify TANs (CD66b, CD15 and cell morphology by H&E stain) may explain contradictory results in prognostic studies of TANs, it is desirable that an optimal combination of markers (i.e. morphology with immunohistochemistry for CD66b) be used in future studies.

Recent studies concerning the role of TANs in human colorectal carcinomas merit further discussion. Berry et al. [91] using morphology and manual counting of TANs in colorectal carcinomas, obtained similar results to ours performed on gastric carcinomas. In particular, they show that in women there was a trend towards better overall survival in all patients with high TAN counts suggesting a potential for different roles for TANs in men and women with colorectal carcinoma [91]. Moreover, higher TAN counts in Stage II patients are associated with a nearly 3-fold increase in overall survival compared to patients with low TAN [91]. The favorable prognostic significance of TANs in colorectal carcinomas is confirmed in two recent studies using CD66b immunohistochemistry as a neutrophil marker. Wikberg et al. [47] have studied the prognostic role of infiltrating neutrophils at different intratumoral subsites including the invasive front, the center of the tumor, and in the tumor epithelium of colorectal carcinomas. Expression of the neutrophil marker CD66b was assessed by immunohistochemistry in 448 archival human tumor tissue samples from patients surgically resected for colorectal carcinomas. They found that high infiltration of CD66b-positive cells in the tumor front is a favorable prognostic factor in stages I-II colon cancers [47]. Galdiero et al [93] used disease-specific and disease-free survival as their endpoints and found that the positive prognostic effect for high TAN counts extended to patients with all stages of disease. TAN density dramatically decreases in Stage IV patients as compared to Stage I-III. They

showed that prognostic significance of TANs can be influenced not only by clinical stage but also 5-fluorouracil (5-FU)-based chemotherapy. In particular, higher TAN density was associated with better response to 5-FU-based chemotherapy [93]. Thus, assessment of TAN infiltration may not only be useful for prognostic informations, but may also have important therapeutic implications, in particular for identifying patients likely to benefit from 5-FU-based chemotherapy. I would like to take the opportunity to stress the *favorable prognostic significance of TANs in gastrointestinal cancer* [82,91-94,100] and its evaluation using rigorous quantitative methodology.

9. Conclusions

Understanding the role of TANs in gastric carcinomas remains incomplete, but studies continue to accumulate. What is certain is that neutrophils are not accidentally present in the tumor stroma, rather they play an active role in tumor growth. Previous studies have suggested positive or negative correlation with patient prognosis. In part, these conflicting results may be due to different methods (morphology vs immunohistochemistry) in quantifying TANs. At the same time, experimental studies reveal antitumoral (N1 phenotype) and protumoral (N2 phenotype) functions of TANs. These data should not necessarily be interpreted as demonstration of two distinct subpopulations of TANs. It is also possible that protumoral and antitumoral neutrophils are two extremes of a spectrum of a sole functional state. Based on recent literature and our own data, this neutrophil functional plasticity correlates strictly with clinicopathological parameters such as gender, tumor stage, intra/peritumoral localization of TANs, and response to chemotherapy. In Table 2, we summarize studies concerning the role of TANs in correlation with clinicopathological parameters. These data suggest that, in particular conditions,TANs represent an antitumoral mechanism that should to be documented in routine pathology and promoted therapeutically. It would also be desirable for pathologists to be involved more and more in determining the type of TAN infiltrate, thereby providing diagnostic and prognostic information as well as suggesting appropriate immune-chemotherapies for each patient.
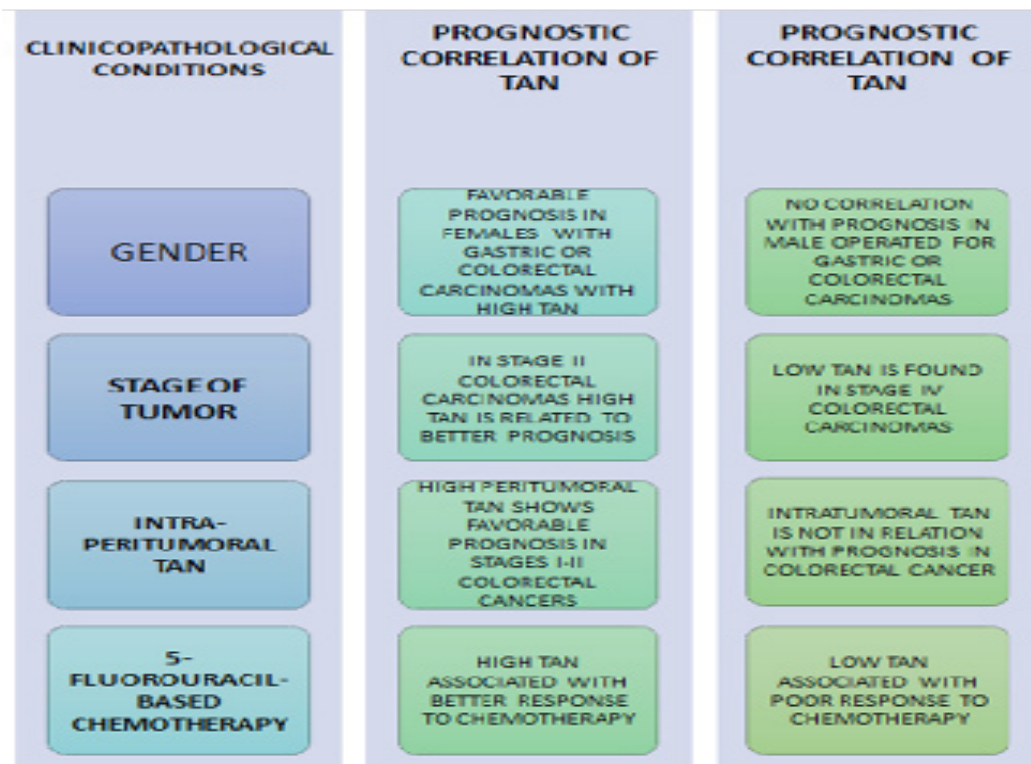
10. Tables

Table 1a: Cell-in-cell structure in both physiological and pathological (non-neoplastic) conditions

EMPERIPOLESIS	EFFEROCYTOSIS
Migration of a cell in the cytoplasm of another	Engulfment of apoptotic cells by macrophages, dendritic cells or adjacent epithelial/mesenchymal cells
During emperipolesis migrating and host cell remain viable	Removal of apoptotic cell commonly occurs in non-neoplastic tissue

Table 1b: Cell-in-cell structure in tumors.

ENTOSIS	XENO-CANNIBALISM
Engulfment of a tumor cell in the cytoplasm of another tumor cell	Phagocytosis of an inflammatory cell by a tumor cell
During entosis engulfed cell is degraded by lysosomal enzymes	During cannibalism engulfed inflammatory cell shows apoptosis

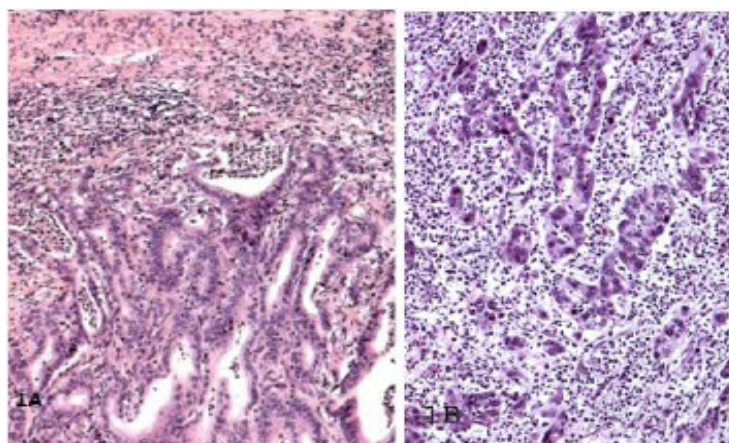**Table 2:** Relationship between clinicopathological factors and bipolar prognostic role of TAN.

CLINICOPATHOLOGICAL CONDITIONS	PROGNOSTIC CORRELATION OF TAN	PROGNOSTIC CORRELATION OF TAN
GENDER	FAVORABLE PROGNOSIS IN FEMALES WITH GASTRIC OR COLORECTAL CARCINOMAS WITH HIGH TAN	NO CORRELATION WITH PROGNOSIS IN MALE OPERATED FOR GASTRIC OR COLORECTAL CARCINOMAS
STAGE OF TUMOR	IN STAGE II COLORECTAL CARCINOMAS HIGH TAN IS RELATED TO BETTER PROGNOSIS	LOW TAN IS FOUND IN STAGE IV COLORECTAL CARCINOMAS
INTRA-PERITUMORAL TAN	HIGH PERITUMORAL TAN SHOWS FAVORABLE PROGNOSIS IN STAGES I-II COLORECTAL CANCERS	INTRATUMORAL TAN IS NOT IN RELATION WITH PROGNOSIS IN COLORECTAL CANCER
5-FLUOROURACIL-BASED CHEMOTHERAPY	HIGH TAN ASSOCIATED WITH BETTER RESPONSE TO CHEMOTHERAPY	LOW TAN ASSOCIATED WITH POOR RESPONSE TO CHEMOTHERAPY

11. Figures

Figure 1
A: TANs are mainly seen at the front of neoplastic tissue. H&E X 100
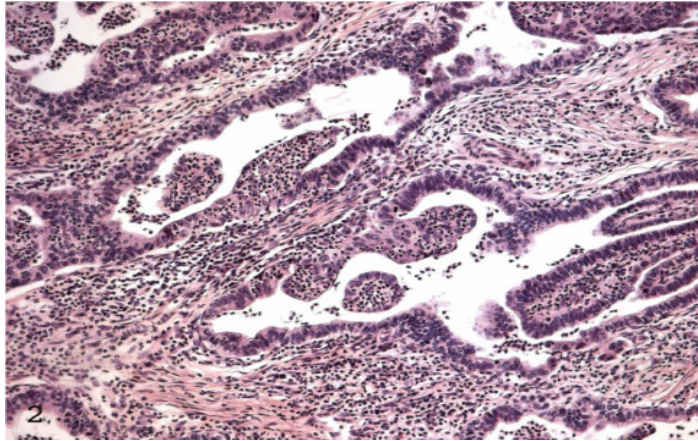B: Numerous TANs in the center of tumor. H&E X 100

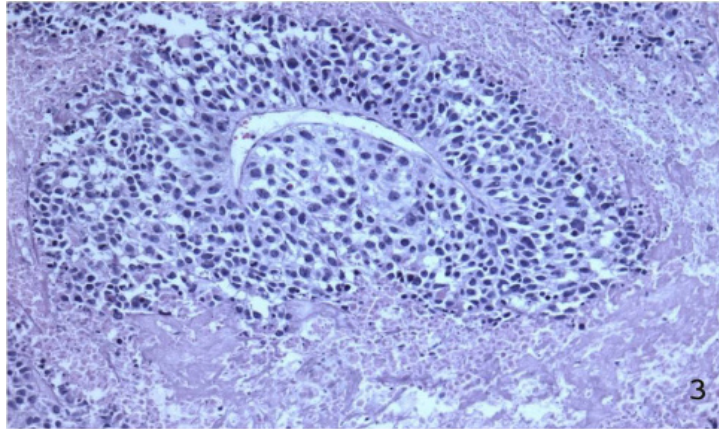**Figure 2:** Gastric adenocarcinoma with numerous intraepithelial neutrophils. H&E X 100

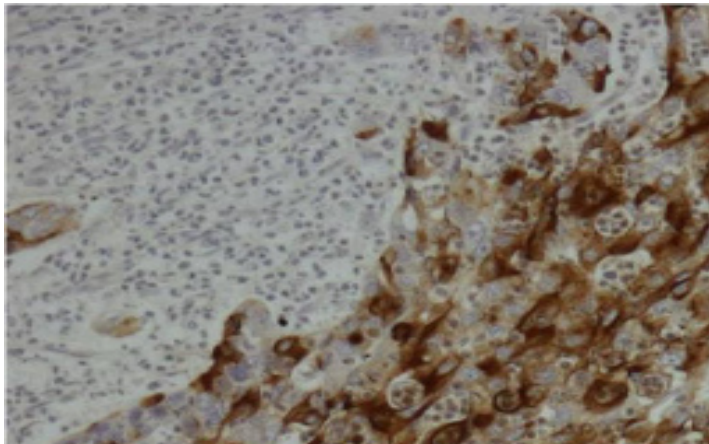**Figure 3:** Large areas of tumor necrosis without neutrophil infiltration. H &E x 100

Figure 4: Strong MUC1 immunoreactivity in the cytoplasm of tumor cells. Note the presence of numerous TANs. X 200
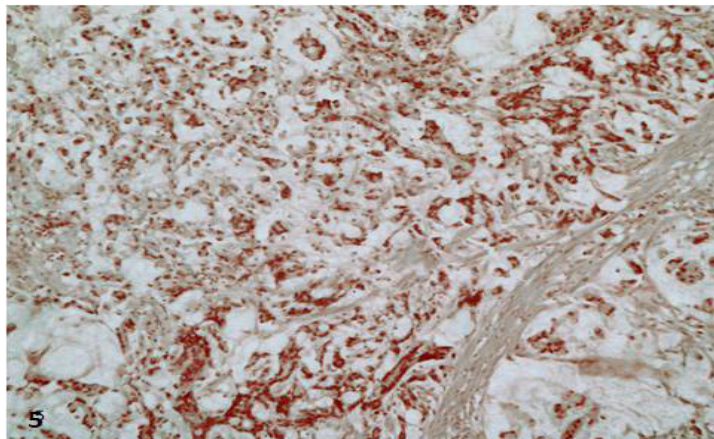

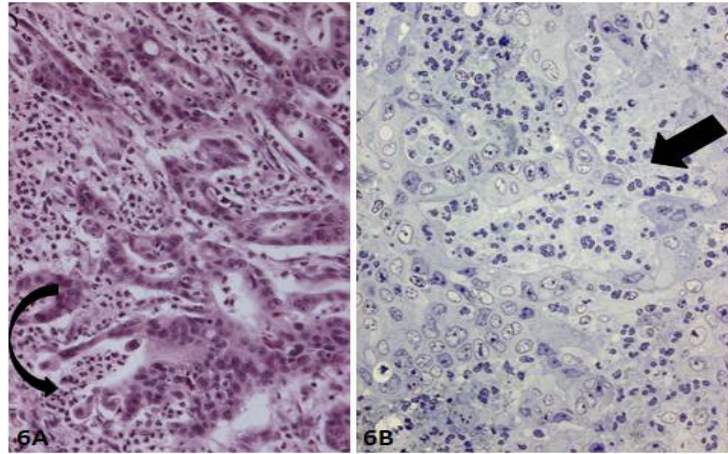**Figure 5:** Mucinous adenocarcinoma of the stomach. Note the absence of neutrophils. H&E X100

Figure 6A: Neutrophil transepithelial migration associated with focal neoplastic gland disruption (curved arrow). H&E X 10 B: Semi-thin section showing numerous TANs and break in continuity of adenocarcinoma gland (arrow). Giemsa X 200
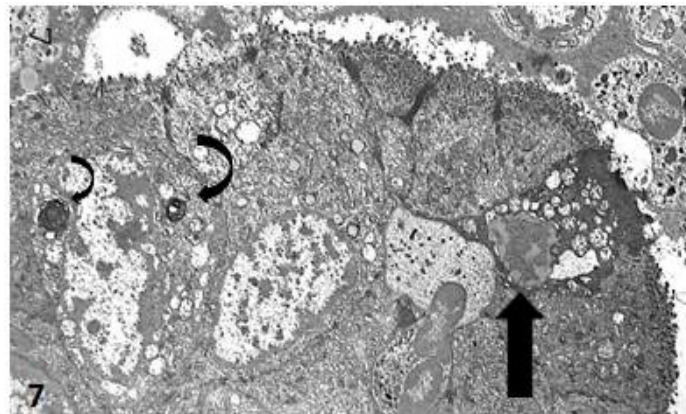

Figure 7: Neutrophil in contact with an adenocarcinoma cell showing chromatin condensation, loss of microvilli, enlarged mitochondria, and dilatation of nuclear envelope (arrow). Note some adjacent tumor cells containing autophagic vacuoles in their cytoplasm (curved arrows). X 8000
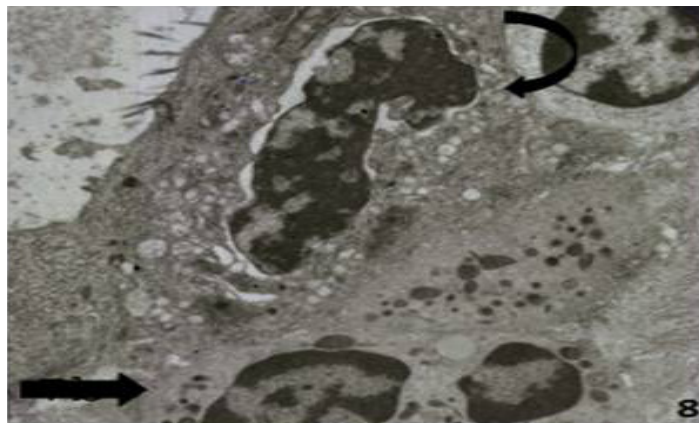

Figure 8: Neutrophils are near adenocarcinoma cell showing convoluted nucleus, marked chromatin condensation, dilatation of nuclear envelope. X 8 000
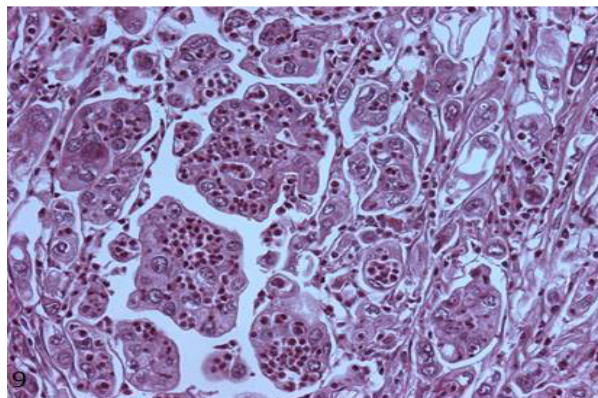

Figure 9: Micropapillary carcinoma of the stomach. Tumor cells exhibit xeno-cannibalism of neutrophils. H&E X 100
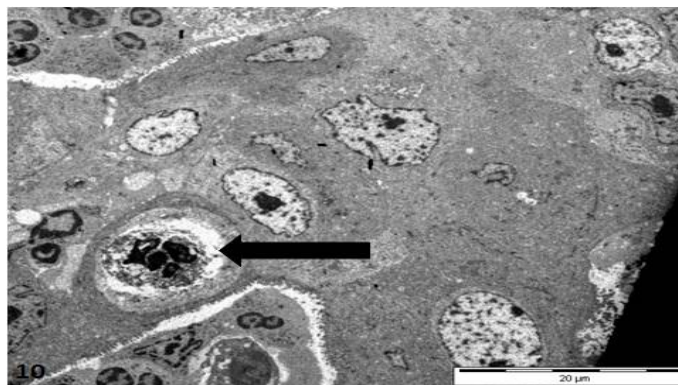
Figure 10: Tumor cell xeno-cannibalism of a neutrophil showing late apoptotic changes. X 4 000

12. References

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