APMB - Atti della Accademia Peloritana dei Pericolanti
Classe di Scienze Medico Biologiche
Vol. 114 (1) 2026

ISSN 1828-6550

DOI: 10.13129 / 1828-6550 / APMB.114.1.2026.SD1

Scholarly Dialog SD1 (1- 20)

Tumor-associated neutrophils in gastric carcinoma: clinicopathological context and favorable prognosis in women

Rosario Caruso, Giovanni Tuccari, Antonio Ieni, Cosimo Inferrera

Department of Human Pathology of Adult and Developmental Age "Gaetano Barresi", Section of Pathological Anatomy, University of Messina, Italy

Abstract

Neutrophils constitute important effectors of innate immunity, yet their role in malignancy remains controversial. Experimental studies have proposed a dichotomous model of tumor-associated neutrophils (TANs), distinguishing antitumoral N1 from protumoral N2 phenotypes. However, this framework may underestimate the clinicopathological context in which TANs operate in human tumors. This narrative review examines the clinicopathological aspects of TANs in gastric carcinoma. Our prior multivariate Cox analysis demonstrated a significant interaction between neutrophil-rich gastric cancer and sex, indicating a favorable prognostic effect solely in women, and subsequently confirmed by two independent studies. Additionally, our histological analysis showed that TAN density was inversely correlated with Goseki Groups II and IV, subtypes of gastric cancer that are distinguished by increased mucin production. TANs are prevalent in Goseki Group I carcinomas, which are characterized by extensive neutrophil infiltration into neoplastic glands. This infiltration is correlated with pseudobudding and near-complete glandular disruption, which are indicative of an antitumoral function. On the other hand, intraepithelial TAN infiltration in gastric micropapillary carcinoma is associated with tumor cell phagocytosis (cannibalism) of neutrophils, indicating a protumoral interaction. These findings provide support for a contextual approach to TANs in gastric cancer that takes into account the N1/N2 paradigm in addition to sex, histological subtype, mucin production, and tumor microenvironment

Key words: Gastric carcinoma; tumor-associated neutrophils (TANs); prognosis; histopathology

Corresponding Author: Rosario Caruso - rocaruso@unime.it

Introducing Member: Cosimo Inferrera

Introduction

Gastric carcinoma is a heterogeneous neoplasm characterized by epidemiological, morphological, molecular, and immunological variability. This complexity is reflected in several classification systems, including Laurén (1), Goseki (2), the World Health Organization (WHO) (3), and The Cancer Genome Atlas (TCGA) (4).

The Laurén classification (1) distinguishes intestinal-type tumors, which are identified by gland-forming neoplastic structures from diffuse-type tumors, which are composed of solitary cells or poorly cohesive clusters lacking glandular differentiation.

Gastric carcinomas are further categorized by tubular differentiation and intracellular mucin content using the Goseki classification (2): Group I (good tubular differentiation, poor intracellular mucin), Group II (good tubular differentiation, abundant intracellular mucin),

Group III (poor tubular differentiation, poor intracellular mucin), and Group IV (poor tubular differentiation, abundant intracellular mucin).

The WHO classification (3) identifies four main histological categories: tubular and papillary adenocarcinomas, that correspond to the Laurén intestinal type; mucinous adenocarcinoma; poorly cohesive carcinoma, corresponding to the Laurén diffuse type; and various rare histological variants.

At the molecular level, TCGA (4) distinguishes four major subtypes of gastric carcinoma: Epstein–Barr virus-associated, microsatellite instability, chromosomal instability, and genomically stable tumors. These groups exhibit morphological associations; the genomically stable subtype is more frequently linked to diffuse-type morphology, while chromosomal instability is more frequently linked to intestinal-type histology.

The predominant leukocytes in peripheral blood are neutrophils, which are essential for innate immunity, particularly in combating bacteria and fungi (5–6). Additionally, they support immunological responses against parasites (7–8) and viruses (9). Neutrophils are equipped with four distinct types of secretory compartments: azurophilic or primary granules (markers: myeloperoxidase and CD63), specific or secondary granules (markers: lipocalin 2, lactoferrin, and CD66b), gelatinase or tertiary granules (markers: gelatinase B and CD11b) (10-11).

Tumor-associated neutrophils (TANs) have garnered more attention in recent years due to their potential therapeutic relevance and prognostic significance in human malignancies. TANs remain difficult to investigate in human tumors because of their short life span, fragility, and low transcriptional activity (12). Consequently, much of the current functional knowledge regarding TANs has been derived from experimental animal models. Fridlender et al. (13) provided seminal evidence for a polarized pattern of TAN activation, proposing an N1/N2 paradigm partly analogous to macrophage and T-cell polarization. In this model, N1 neutrophils exert antitumoral effects, whereas N2 neutrophils promote tumor progression. The N1/N2 paradigm has since been widely adopted because it offers a concise framework for interpreting neutrophil functional heterogeneity, although most supporting evidence has been derived from murine models (14). Moreover, in contrast to TAMs, no reliable marker panel has been established for these two phenotypes, limiting the possibility of directly assessing TAN polarization by immunohistochemistry in human tumor tissues (15).

This narrative review offers a histopathological reinterpretation of TANs in gastric carcinoma. Unlike previous reviews, which have mainly focused on neutrophil recruitment and on the conventional N1/N2 model of antitumoral versus protumoral functions, the present review examines TANs within their clinicopathological context. Drawing on our institutional

experience, we describe histopathological findings consistent with an antitumoral role of neutrophils, particularly in pseudobudding observed in selected histological subtypes of gastric carcinoma. We also highlight intraepithelial TAN infiltration associated with tumor cell cannibalism of neutrophils in gastric micropapillary carcinomas, a pattern suggestive of a protumoral interaction. In addition, we revisit the sex-specific prognostic significance of TANs in resected gastric carcinoma, including independent studies supporting a favorable effect in women. Finally, we briefly discuss emerging preclinical strategies that utilize neutrophil recruitment and tumor infiltration as a mechanism for anticancer drug delivery. Taken together, these observations support a contextual interpretation of TANs in which sex, histological subtype, tumor architecture, and tumor microenvironment are integrated with the N1/N2 paradigm.

Identification of tumor-associated neutrophils

Several methods have been used to identify TANs in human tumors. In routine histopathology, TANs can usually be recognized on hematoxylin and eosin (H&E) -stained sections by their multilobed nuclei and distribution within stromal, intraepithelial, and intraglandular compartments (16-17).

Immunohistochemistry can identify TANs more specifically, using markers such as myeloperoxidase, CD15, CD177, and CD66b. However, none is entirely specific. As pointed out by Galdiero et al. (18), myeloperoxidase immunostaining is not entirely specific for neutrophils, since it may also label monocytes and immature macrophages. Similarly, CD15 may be expressed by eosinophils, some monocytes, and, in certain cases, tumor cells. This may interfere with assessment of intratumoral neutrophils, although peritumoral evaluation is less affected (18). CD177, involved in neutrophil–endothelial adhesion and migration, has been proposed as a useful TAN marker (19). CD66b is widely used as a granulocyte activation marker, but eosinophil expression may reduce its specificity, especially in eosinophil-rich tumors (20).

Thus, no single immunohistochemical marker is sufficient to characterize neutrophil populations within human tumors. Multiple markers, in conjunction with serial H&E-stained sections as a morphological reference, may be necessary to distinguish TANs from eosinophils and estimate their relative proportions.

Assessment of tumor-associated neutrophil density

TAN density can be assessed manually by counting cells in non-overlapping high-power fields, usually at 400× magnification, or by automated image analysis. Automated or computer-based approaches generally provide more reproducible immune-cell density estimates than visual

semiquantitative evaluation (21).

In our series, stromal neutrophils were evaluated semiquantitatively on H&E-stained sections by counting cells in 20 non-overlapping high-power fields at 400× magnification, corresponding to 0.08 mm² per field (22). Cases were then dichotomized using the median TAN count observed in the series as the cut-off; this corresponded to a threshold of more than 10 neutrophils across 20 high-power fields (22).

Tumors exceeding this threshold were operationally defined as neutrophil-rich gastric carcinomas, whereas tumors with 10 or fewer neutrophils were classified as having minor neutrophilic infiltration (22).

Neutrophil-rich gastric carcinomas: the importance of histological context

Our studies indicate that neutrophil-rich gastric carcinomas represent a limited subset of gastric tumors, with reported frequencies of 7.6%–15.5% (22-23). Prominent TAN infiltration is therefore not a uniform feature but is associated with specific histopathological backgrounds. The Goseki classification (2) may be particularly informative because it categorizes gastric adenocarcinomas according to glandular differentiation and mucin production. Goseki Groups I and III, which share low intracellular mucin content, tend to show extensive TAN infiltration (Figures 1A and 2A). By contrast, Groups II and IV, characterized by abundant mucin production, usually show scant or absent neutrophilic infiltration (Figures 1B and 2B). These differences suggest that TAN recruitment may be limited, at least partly, by abundant extracellular mucin in Goseki Groups II and IV. According to research conducted on mucinous colorectal adenocarcinomas (24-25), extracellular mucin may function as a physical barrier, inhibiting the infiltration of granulocytes and effector T cells and reducing the penetration of antitumor agents. A similar model may apply to mucin-rich gastric carcinomas. Figure 2B demonstrates that extracellular mucin lakes encircle signet-ring cells, thereby separating neoplastic cells from the inflammatory infiltrate and potentially impeding direct neutrophil–tumor cell interactions. These observations indicate that the significance of TANs cannot be interpreted independently of the architectural and microenvironmental setting. Relevant factors include extracellular mucin abundance, glandular organization, and growth pattern, whether glandular, solid, poorly cohesive, micropapillary, or mucinous. This histopathological perspective remains underrepresented in reviews focused mainly on TAN molecular biology and the N1/N2 paradigm.

Focused prognostic studies stratified by Goseki group are therefore needed. Comparisons between Group I and II tumors, and between Group III and IV tumors, may clarify whether mucin production and tumor architecture influence TAN recruitment and prognostic significance in gastric carcinoma.

Fig. 1. Goseki Group I gastric adenocarcinoma showing intraglandular neutrophil exudation. Neutrophils are present both in the tumor stroma and within neoplastic glands showing cribriform architecture. Segmental disruption of the glandular lining is evident. (A, H&E, original magnification 100X). Goseki Group II gastric adenocarcinoma. Tumor cells show clear cytoplasm due to abundant intracellular mucin. TANs and segmental gland disruption are not appreciable in this field. (B, H&E, original magnification, 100X).

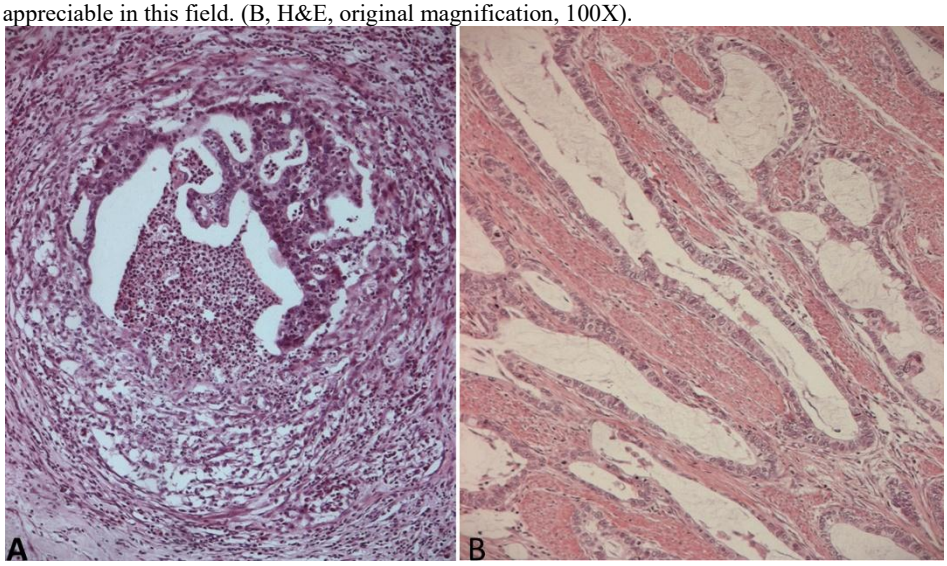
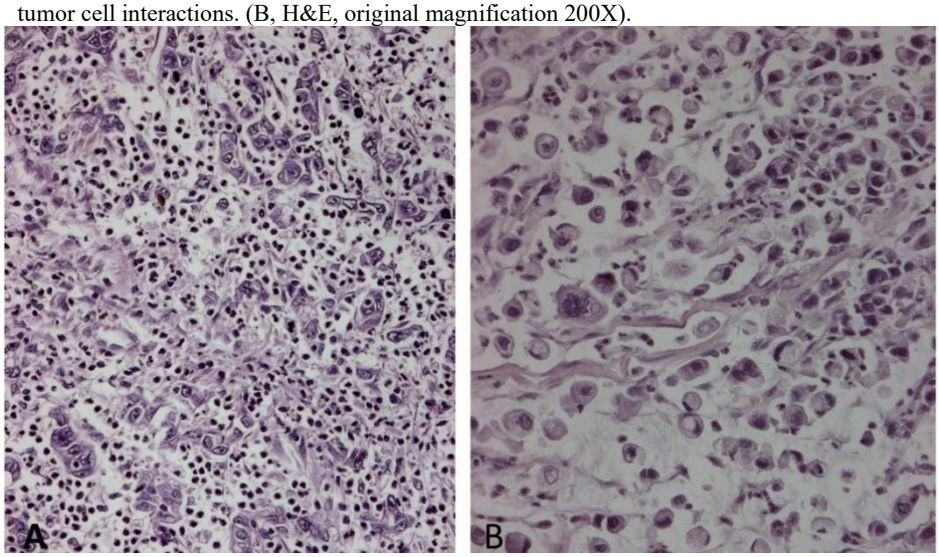


Fig. 2. Goseki Group III gastric carcinoma showing single tumor cells and small tumor clusters associated with marked neutrophilic infiltration. (A, H&E, original magnification 100X). Goseki Group IV gastric carcinoma showing signet-ring cells embedded in abundant extracellular mucin pools. Tumor-associated neutrophils are virtually absent, consistent with the hypothesis that extracellular mucin may act as a physical barrier to direct neutrophil–tumor cell interactions. (B, H&E, original magnification 200X).



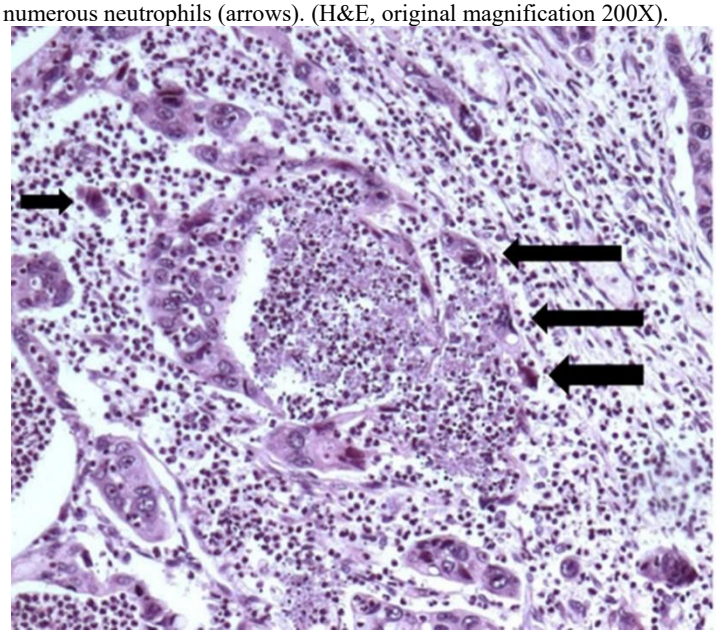
Pseudobudding: neutrophil-associated glandular disruption does not represent true tumor budding

Cancer cells can invade individually, after loss of cell-cell adhesion, or collectively, while retaining intercellular cohesion (26). Among the best-studied histopathological manifestations of invasion are tumor budding (27-29) and poorly differentiated clusters (27, 30-33), routinely assessed in colorectal cancer and investigated in other epithelial malignancies. Tumor budding is

defined as single tumor cells or clusters of up to four cells, whereas poorly differentiated clusters consist of five or more tumor cells lacking gland formation (29). Tumor budding is now recognized as a strong predictor of lymph node metastasis and cancer-related death in several epithelial cancers (28).

Tumor budding should not be confused with pseudobudding, a recently proposed histopathological term describing a marked inflammatory reaction at the invasive front, where single tumor cells and small clusters are observed in close proximity to fragmented glands (34). Haddad et al. (34) established that tumor budding and pseudobudding are biologically distinct entities with different morphological, immunohistochemical, microenvironmental, and biomolecular features. In our experience, pseudobudding is frequent in Goseki Group I gastric carcinoma and is characterized by massive neutrophil migration into neoplastic gland lumina (Figures 1A and 3).

Fig. 3. Goseki Group I gastric adenocarcinoma showing pseudobudding. Near-complete disruption of the glandular architecture is evident, with isolated necrotic tumor cells and small tumor cell clusters surrounded by numerous neutrophils (arrows). (H&E, original magnification 200X).



Intraglandular neutrophil accumulation is associated with segmental glandular disruption, ranging from focal breaches involving a few adjacent adenocarcinoma cells (Figure 4) to near-complete gland disruption (Figure 3). Single tumor cells or small clusters of up to four cells remain close to disrupted glands and are often surrounded by neutrophil microabscesses (Figures 3 and 4). Atypical mitoses may be evident in residual adenocarcinoma cells (Figure 4). Our previous studies showed that massive intraglandular neutrophil migration is spatially associated with non-apoptotic tumor cell injury (35-38). Dying tumor cells do not show apoptotic changes, such as chromatin condensation, cell contraction, or the formation of apoptotic bodies (37-38). Furthermore, they are TUNEL-negative and display ultrastructural signs of non-apoptotic cellular damage (Figure 5) (37-38).

Fig. 4. Early phase of pseudobudding. Intraglandular neutrophil migration affects a small group of adenocarcinoma cells, leading to a focal break in the glandular lining (arrowhead). A neutrophil microabscess is present within the neoplastic gland, together with a necrotic tumor cell (curved arrow). Some residual adenocarcinoma cells exhibit atypical mitoses (arrows). Numerous neutrophils are also observed in the tumor stroma. (H&E, original magnification 200X).

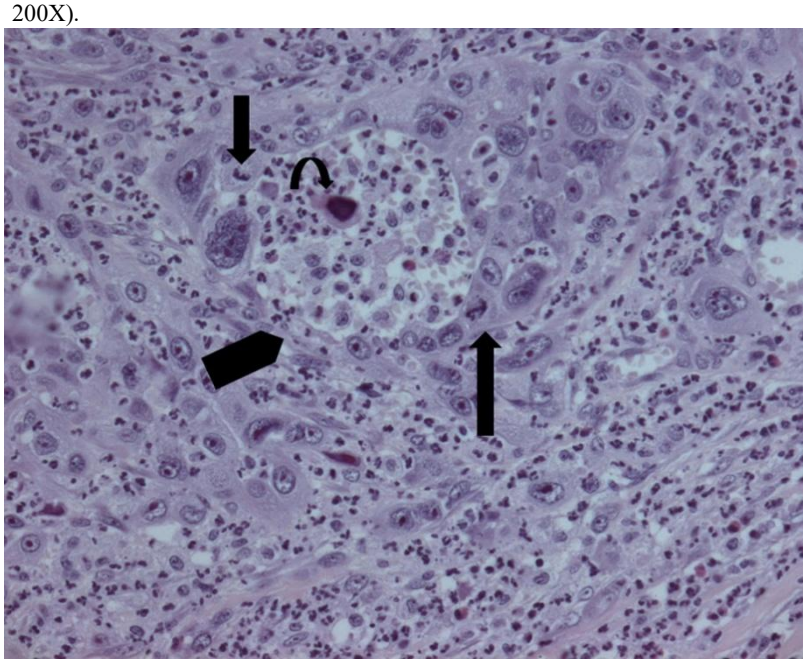
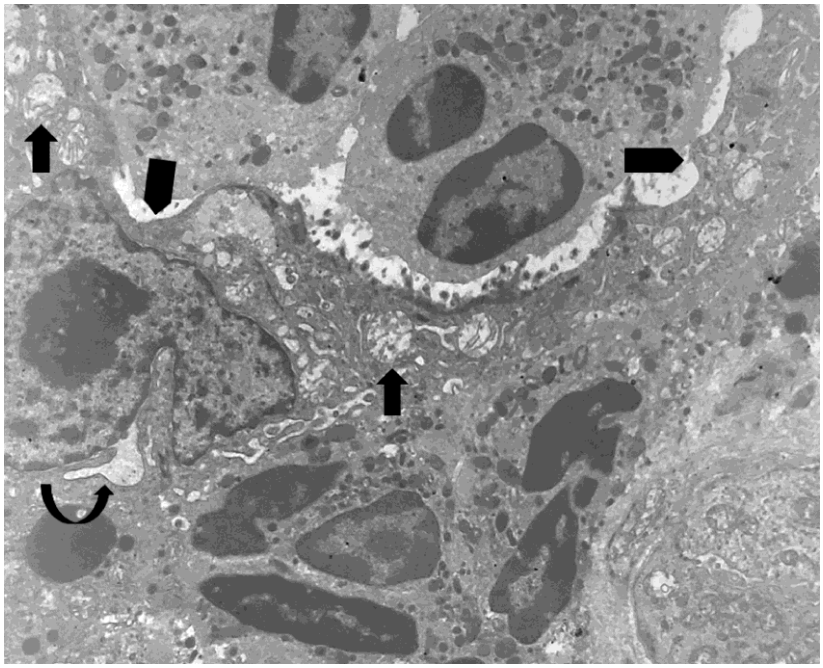


Fig. 5. Ultrastructural features of intraepithelial neutrophil migration in a Goseki Group I gastric adenocarcinoma, with additional neutrophils visible within the glandular lumen. Tumor cells directly contacted by intraepithelial neutrophils show marked ultrastructural injury, including patchy chromatin condensation, dilatation of the nuclear envelope (curved arrow), mitochondrial swelling (arrows), and loss of microvilli (arrowheads). The absence of apoptotic body formation and complete nuclear fragmentation supports a non-apoptotic type of tumor cell injury.



In true tumor budding, by contrast, single tumor cells or small clusters are usually located in the stroma at some distance from neoplastic glands and are not surrounded by neutrophil microabscesses

(28-29). Pseudobudding areas show significantly higher nuclear Ki-67 expression than tumor budding, whereas lack of Ki-67 expression and absence of mitotic figures are commonly reported features of true budding (34).

Tumor budding areas display a phenotypic shift relative to the tumor bulk, with acquisition of migratory properties and expression of epithelial-mesenchymal transition-related markers (34). In contrast, pseudobudding lacks epithelial-mesenchymal transition features. These findings indicate that pseudobudding is not true invasive tumor budding, but rather glandular fragmentation-associated scattering of tumor cells secondary to neutrophil-associated glandular disruption (34). True budding reflects activation of tumor cell programs related to migration and invasion. Molecular alterations associated with tumor budding include activation of the c-MET/HGF and PDGF pathways, promoting invasion, metastasis, and angiogenesis (34). By contrast, pseudobudding has been associated with DNA damage repair pathways and host defense peptides, possibly reflecting neutrophil-mediated tumor epithelial injury in areas of near-complete gland disruption (34). Current guidelines recommend excluding pseudobudding from routine tumor budding assessment because inclusion may lead to an erroneously high tumor bud score (28-29). In difficult cases, Ki-67 immunostaining may help distinguish pseudobudding from true budding, since proliferative activity is usually retained in pseudobudding but absent or markedly reduced in true tumor budding (34). The main morphological and immunohistochemical features useful for distinguishing tumor budding from pseudobudding are summarized in table 1.

Tab. 1. Histopathological and immunohistochemical criteria for the differential diagnosis between tumor budding and pseudobudding.

Feature	Tumor budding	Pseudobudding
Single tumor cells or clusters of up to four tumor cells	Present	Present
Spatial distribution	Stromal buds at the invasive front, usually separated from parent glands	Single cells or small clusters adjacent to disrupted or fragmented glands
Relationship with neutrophils	Not surrounded by neutrophil microabscesses	Closely associated with neutrophil microabscesses
Intraglandular neutrophil migration	Absent	Present
Glandular disruption or fragmentation	Absent in budding areas	Present
Atypical mitoses	Absent	May be present in residual glandular tumor cells
Ki-67 immunoreactivity	Absent or markedly reduced	Retained or increased nuclear expression
Epithelial–mesenchymal transition-related features	Present	Absent
Main biological interpretation	Tumor cell-intrinsic invasive process	Glandular fragmentation-associated scattering of tumor cells

No large series has specifically investigated the prognostic role of pseudobudding in gastric carcinoma. Further studies are needed to evaluate its prognostic significance in large cohorts of patients with gastric carcinoma.

Definitions of tumor budding, poorly differentiated clusters, and micropapillary carcinoma

Micropapillary carcinoma was first described by Siriaunkgul and Tavassoli (39) as a rare subtype of invasive ductal carcinoma of the breast characterized by pseudopapillary tumor cell clusters lacking fibrovascular cores and surrounded by clear stromal spaces. This distinctive architecture has subsequently been recognized in several organs, including lung, urinary bladder, pancreas, colorectum, and stomach (40).

Immunohistochemistry confirmed aberrant redistribution of apical/luminal markers, including MUC1, EMA, CD10, and villin, to the outer stromal-facing surface of micropapillary clusters (40). Ultrastructural studies by Luna-Moré et al. (41) and by our group (42) in breast micropapillary carcinoma demonstrated that the microvilli of the cell surface are oriented toward the stromal-facing surface of tumor cells and not toward the lumen of the neoplastic glands

These observations suggest that micropapillary growth arises from reversal of tumor cell polarity, whereby the stromal-facing surface acquires apical/luminal immunophenotypic features (40). This “inside-out” pattern is now regarded as a defining diagnostic hallmark of micropapillary carcinoma (40).

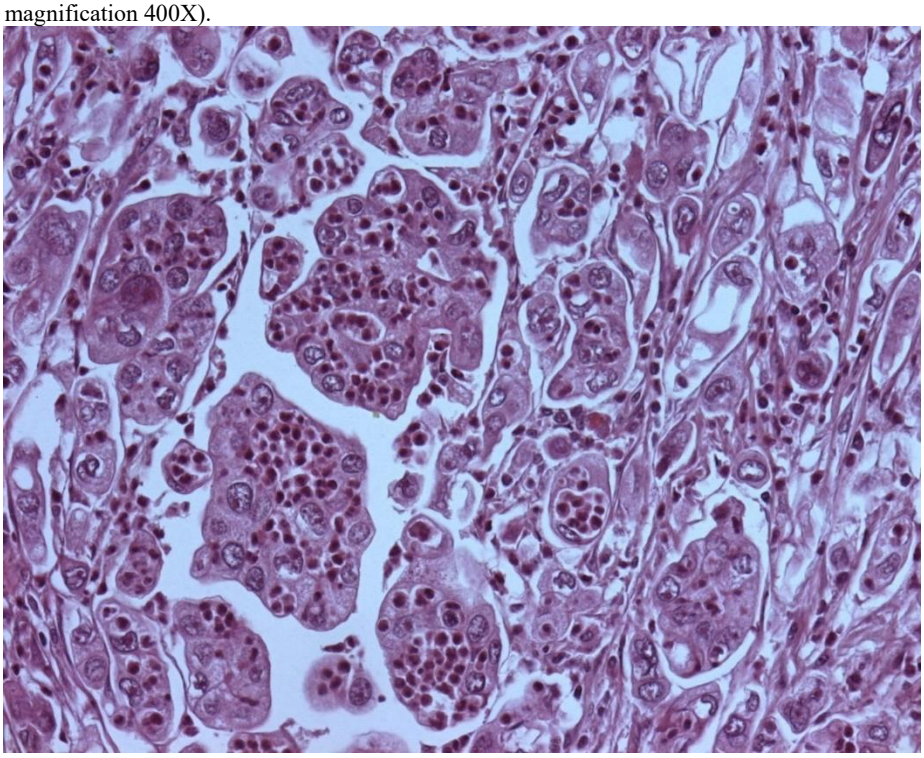
Our study group (43) reported that reversed MUC1 expression and loss of E-cadherin are also observed in poorly differentiated clusters in colorectal carcinoma. These findings support the view, shared by several authors (44-46), that the distinction between micropapillary clusters and poorly differentiated clusters may be difficult and that both patterns could represent closely related manifestations of the same biological phenomenon.

Abundant intraepithelial TAN infiltration has been described in selected micropapillary carcinomas of the pancreas and ampullo-pancreatobiliary region (47-49), colorectum (50-51), and stomach (52-53), suggesting that this architectural pattern may provide a permissive setting for close neutrophil–tumor cell interactions. Our study group (53) reported five cases of gastric micropapillary carcinoma with numerous TANs showing a distinct infiltration pattern. TANs were predominantly intraepithelial within micropapillary clusters and only rarely formed extracellular stromal microabscesses (Figure 6). This contrasts with pseudobudding, where neutrophil microabscesses accompany glandular disruption. Some intraepithelial neutrophils were enclosed within cytoplasmic vacuoles of tumor cells and showed apoptotic features, including pyknotic nuclei, TUNEL staining and caspase-3 immunoreactivity (53). These findings support

tumor cell cannibalism of neutrophils, defined as the ability of tumor cells to engulf not only neighboring neoplastic cells but also immune cells (54-57).

Tumor cell cannibalism of neutrophils must be distinguished from other cell-in-cell phenomena, such as emperipolesis and entosis (57). Emperipolesis refers to the passage of one cell through the cytoplasm of another without significant damage to either the host cell or the internalized cell (58-59). This mechanism is unlikely in our cases, because the neutrophils enclosed within micropapillary carcinoma cells showed clear apoptotic changes. Entosis, a term introduced by Overholtzer et al. (60), may morphologically resemble emperipolesis but is typically associated with a non-apoptotic fate of the engulfed cell (61), in contrast to the apoptotic features observed in our micropapillary cases. These findings may therefore be interpreted as morphological evidence suggestive of a protumoral interaction between TANs and micropapillary carcinoma cells. Tumor cell cannibalism of neutrophils may represent a form of tumor cell “feeding” activity, potentially supporting tumor cell survival in poorly vascularized microenvironments (62–65). Thus, TANs may interact with gastric carcinoma cells through distinct histopathological patterns, ranging from pseudobudding in Goseki Group I tumors to tumor cell cannibalism of neutrophils in micropapillary carcinomas, possibly corresponding to antitumoral and protumoral roles, respectively.

Fig. 6. Gastric micropapillary carcinoma showing micropapillary clusters infiltrated by numerous neutrophils. Collections of neutrophils are contained within cytoplasmic vacuoles of tumor cells, consistent with tumor cell cannibalism of neutrophils. (H&E, original magnification 400X).



Systemic versus intratumoral neutrophils in gastric carcinoma

Peripheral neutrophilia and an elevated neutrophil-to-lymphocyte ratio have been widely associated with adverse clinical outcomes in patients with solid tumors, as shown by large meta-analyses and umbrella reviews (66-68). However, neutrophil-to-lymphocyte ratio is a systemic inflammatory index influenced by tumor-independent variables, including infections, lifestyle factors, and cardiovascular or metabolic comorbidities, and cannot be assumed to reflect the biological role of neutrophils within tumor tissue (69-71). This distinction is crucial. Systemic neutrophil-related markers often correlate with adverse outcome, whereas intratumoral neutrophils may have a different, context-dependent significance, as suggested by our original study (22) and subsequently confirmed by Clausen et al. (72) and Quaas et al. (73) in sex-stratified prognostic models of gastric and upper gastrointestinal adenocarcinomas.

The Messina study group and subsequent prognostic confirmations

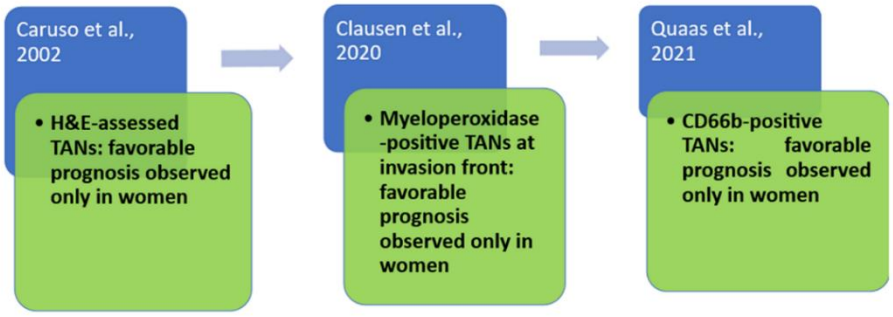
In our 2002 study (22), the prognostic significance of TANs was investigated in a series of 273 patients with advanced gastric carcinoma who underwent gastrectomy at Cremona Hospital, Lombardy, Italy, between 1990 and 1995 and were followed for 5 years. Tumors were classified as neutrophil-rich (n = 76; >10 TANs across 20 high-power fields) or neutrophil-poor (n = 197; ≤10 TANs across 20 high-power fields), according to the semiquantitative criteria described above. Multivariate Cox analysis demonstrated a significant interaction between neutrophil-rich gastric carcinoma and sex, showing that female patients with neutrophil-rich tumors had an approximately 39% reduction in the risk of death, whereas no comparable prognostic effect was observed in men (22).

These findings were later supported by independent studies using sex-stratified models. Clausen et al. (72) assessed TAN density in different tumor compartments using myeloperoxidase immunohistochemistry and digital image analysis on whole-tissue sections and defined tumor areas, reducing observer-related bias. TAN density showed marked interindividual variability and heterogeneous distribution across tumor surface, center, and invasion front. It was associated with intestinal phenotype, tumor grade, and microsatellite status in the tumor center and invasion front. In multivariate analysis, TAN density at the invasion front independently predicted tumor-specific survival only in women. This study therefore supported our observation that the prognostic significance of neutrophilic infiltration in gastric carcinoma is sex-specific and particularly evident at the invasion front (72).

Quaas et al. (73) extended this evidence in a large European cohort of 1118 patients, including 458 gastric and 660 esophageal adenocarcinomas, with both primarily resected and neoadjuvant-treated tumors. TANs were assessed by CD66b immunostaining for quantitative image analysis,

together with morphological evaluation of neutrophils on routine H&E-stained sections by an experienced pathologist. This combined approach provided a morphological check of immunohistochemical quantification. In gastric adenocarcinoma, increased stromal TAN density was associated with favorable prognosis, mainly in the chromosomal instability subtype of the TCGA classification, a molecular subgroup frequently associated with intestinal-type histology according to Laurén (1,4). Notably, the favorable prognostic impact was observed only in women. A similar sex-specific pattern was documented in esophageal adenocarcinoma and in women receiving neoadjuvant therapy. Overall, this study strengthened the evidence that TAN prognostic significance in upper gastrointestinal adenocarcinomas is sex-dependent and extends beyond gastric carcinoma (Figure 7) (73).

Fig. 7. Sex-stratified prognostic models of TANs in gastric cancer. In all three studies, multivariate Cox models showed that the favorable prognostic significance of TANs was observed only in women, despite differences in TAN assessment methods



Sexual dimorphism and the female-specific prognostic significance of TANs

Sex-related differences in neutrophil biology have long been recognized. A classic morphological feature is the drumstick appendage (Barr body), representing sex chromatin related to the inactive X chromosome, visible in a small proportion of female neutrophils on stained peripheral blood smears and absent or exceedingly rare in normal male neutrophils (74-75).

Functional differences are also well documented. In many immunological settings, women show stronger innate and adaptive immune responses than men, but also higher susceptibility to autoimmune disease (76). Neutrophils may contribute to this dimorphism: phagocytic activity has been reported to be greater in females, and sex hormones may modulate apoptosis, and functional activity across the menstrual cycle (76-77). Testosterone is generally immunomodulatory and often immunosuppressive, although experimental studies suggest that it may enhance neutrophil

activation in selected non-infectious inflammatory settings, such as trauma-hemorrhagic shock and burn injury (76-77).

These observations suggest that neutrophil behavior is shaped by sex chromosomes, endocrine regulation, epigenetic mechanisms, and environmental influences (76-77). In gastric carcinoma, this biological background may provide a plausible framework for interpreting the female-specific favorable prognostic significance of TANs observed in our study and confirmed by independent reports (22, 72-73). Based on the recognized sexual dimorphism of neutrophil biology, female neutrophils may differ not only in systemic responsiveness but also, potentially, in local antitumoral activity within the gastric tumor microenvironment (76-77). Current evidence is insufficient to determine whether this sex-specific effect is modulated by menopausal status. Future studies should assess premenopausal and postmenopausal women separately and clarify the contribution of sex hormones to neutrophil recruitment, activation, and tumor cell injury in gastric carcinoma. Other studies yielded divergent results (Table 2).

Tab. 2. Additional prognostic studies of tumor-associated neutrophils in gastric carcinoma without sex-stratified survival analysis.

Main finding	TAN assessment	Methodological note	Reference
Stromal CD15-positive cells associated with unfavorable prognosis	CD15 immunohistochemistry	CD15 not neutrophil-specific; possible bias in TAN quantification	Zhao et al. (81)
Absence of TANs associated with lymph node metastasis in EBV-associated gastric cancer	CD66b and CD8 immunohistochemistry with digital image analysis	EBV-associated gastric cancer subgroup: no sex-stratified analysis	Abe et al. (80)
High TAN density associated with favorable clinicopathological features and improved prognosis	CD66b immunohistochemistry	No sex-stratified analysis	Huang et al. (78)
Increased TAN density associated with favorable prognosis	CD66b immunohistochemistry	No sex-stratified analysis	Zhang et al. (82)

Zhao et al. (81) reported that stromal CD15-positive inflammatory cells were associated with unfavorable prognosis in Asian patients with gastric carcinoma; however, CD15 is not neutrophil-specific and may label monocytes, eosinophils, potentially biasing TAN quantification (73).

By contrast, Zhang et al. (82) used CD66b immunohistochemistry and found that increased tumor-infiltrating neutrophil density was associated with favorable prognosis and predicted benefit from postoperative adjuvant chemotherapy in patients with gastric cancer. However, these prognostic studies did not apply sex-stratified survival models, limiting direct assessment of whether the favorable effect is equally distributed between men and women or mainly driven by female patients.

Therapeutic implications: neutrophils as vehicles for targeted drug delivery in anti-cancer strategies

Neutrophils, pivotal in inflammatory responses in certain tumor types, have attracted growing interest as potential cellular vehicles for targeted drug delivery in anticancer treatment (83). Preclinical studies showed that neutrophils loaded with paclitaxel-containing liposomes inhibited glioma recurrence in mice by migrating to postsurgical inflammatory areas (84). Similarly, human neutrophils loaded with albumin-bound paclitaxel nanoparticles exhibited homing ability in an ectopic gastric cancer model, especially when surgery or radiotherapy was used to promote neutrophil infiltration to tumor sites (85). Other experimental approaches include photosensitizer-laden neutrophils remotely activated for cancer immunotherapy (86) and CAR-neutrophil-mediated delivery of tumor-microenvironment-responsive nanodrugs in glioblastoma models (87*)*. These strategies, however, depend on efficient neutrophil homing to tumor tissue, a process influenced, at least in part, by the local histopathological context. In gastric carcinoma, this consideration is particularly relevant because neutrophil recruitment appears to be influenced by tumor architecture, mucin production, and the inflammatory microenvironment. Therefore, future neutrophil-mediated drug delivery approaches should consider not only molecular targets but also the histopathological conditions that favor TAN infiltration and tumor cell contact.

Limitations of the review

This review has some limitations. First, it is narrative rather than systematic and does not provide a quantitative synthesis of the available evidence. Second, several interpretations are based on histopathological correlations and representative images from institutional material and should therefore be regarded as hypothesis-generating until validated in larger independent series. Third, available prognostic studies are heterogeneous in TAN identification methods, tumor compartments, patient populations, histological and molecular subtypes, and use of sex-stratified models, thereby limiting direct comparability. Finally, because reliable markers for directly identifying N1 and N2 TAN phenotypes in routine human tumor tissues are lacking, functional interpretation remains inferential and must be integrated with clinicopathological and morphological context.

Conclusions

Our original observation on the favorable prognostic significance of TANs was confirmed nearly two decades later by two independent studies showing a sex-specific beneficial effect in women undergoing surgery for gastric carcinoma (22,72–73). Thus, TANs may be associated with a favorable outcome in selected biological contexts, a finding that may remain undetected without sex-stratified analyses.

Recent TAN literature has largely relied on the N1/N2 dichotomy, a framework derived mainly from experimental models and insufficiently attentive to histopathological context. The Goseki classification offers a useful complementary framework because it stratifies gastric carcinomas by mucin production and glandular differentiation (2). Since extracellular mucin may protect neoplastic cells from neutrophils and other immune effectors (24–25), future studies should combine sex-stratified analyses with histotype-oriented stratification. In particular, comparisons between Goseki Groups I and II, and between Groups III and IV, may clarify whether mucin production influences TAN recruitment and modifies their prognostic significance independently of glandular differentiation.

Although preliminary and limited to selected settings, our histopathological data suggest that TAN–tumor cell interactions vary according to tumor architecture and phenotype. Two paradigmatic examples are proposed in this review. The first is pseudobudding, in which the favorable prognostic effect observed in women may be partly related to extensive neutrophil infiltration associated with neoplastic gland disruption (22,72–73). The second is tumor cell cannibalism of neutrophils in gastric micropapillary carcinoma, where intraepithelial neutrophils are engulfed by tumor cells, possibly providing nutritional support and fostering a protumoral environment (53,62–64). These contrasting patterns indicate that the biological significance of TANs may range from a potentially antitumoral pattern in pseudobudding to a potentially protumoral pattern in micropapillary carcinoma, although their precise functional role remains to be fully established.

A more rigorous interpretation of the N1/N2 dichotomy requires integration with clinicopathological variables such as sex, histological subtype, tumor architecture, and mucin production. Without this contextualization, the N1/N2 paradigm risks remaining ambiguous and only partially informative. Many reviews describe TANs as a compilation of apparently contradictory antitumoral and protumoral functions; however, a clinicopathological approach that considers sex, tumor histotype, and architectural context may more accurately define the conditions under which TANs display antitumoral or protumoral activities. This perspective may also inform future neutrophil-based therapeutic strategies, including the use of neutrophils as cellular vehicles for anticancer drug delivery, by identifying the histopathological conditions that favor TAN recruitment and tumor cell contact.

Conflicts of Interest. The Authors declare no conflict of interest

References

1. Laurén, P. (1965). The two main histological types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand. 64:31–49. doi: 10.1111/apm.1965.64.1.31.

2. Goseki, N., Takizawa, T., Koike, M. (1992). Differences in the mode of the extension of gastric cancer classified by histological type: new histological classification of gastric carcinoma. Gut. 33(5):606–612. doi: 10.1136/gut.33.5.606.
3. Carneiro, F., Fukayama, M., Grabsch, H.I., Yasui, W. (2019). Gastric adenocarcinoma. In Digestive System Tumours, 5th ed.; WHO Classification of Tumours Editorial Board; World Health Organization: Lyon, France; Volume 1, pp. 85–95.
4. Cancer Genome Atlas Research Network. (2014). Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 513(7517):202–209. doi: 10.1038/nature13480.
5. Shrestha, S., Hong, C.W. (2023). Extracellular mechanisms of neutrophils in immune cell crosstalk. Immune Netw. 23(5):e38. doi: 10.4110/in.2023.23.e38.
6. Gordon, S. (2016). Phagocytosis: an immunobiologic process. Immunity. 44(3):463–475. doi: 10.1016/j.immuni.2016.02.026.
7. Inferrera, C., Princi, P. (1967). Leishmaniosi viscerale: reperti ultrastrutturali nelle cellule midollari [Visceral leishmaniasis: ultrastructural findings in the bone marrow cells]. Arch De Vecchi Anat Patol. 49(3):757–775.
8. Charmoy, M., Auderset, F., Allenbach, C., Tacchini-Cottier, F. (2010). The prominent role of neutrophils during the initial phase of infection by *Leishmania* parasites. J Biomed Biotechnol. 2010:719361. doi: 10.1155/2010/719361.
9. Ma, Y., Zhang, Y., Zhu, L. (2021). Role of neutrophils in acute viral infection. Immun Inflamm Dis. 9(4):1186–1196. doi: 10.1002/iid3.500.
10. Mollinedo, F. (2019). Neutrophil degranulation, plasticity, and cancer metastasis. Trends Immunol. 40(3):228–242. doi: 10.1016/j.it.2019.01.006.
11. Othman, A., Sekheri, M., Filep, J.G. (2022). Roles of neutrophil granule proteins in orchestrating inflammation and immunity. FEBS J. 289(14):3932–3953. doi: 10.1111/febs.15803.
12. Hedrick, C.C., Malanchi, I. (2022). Neutrophils in cancer: heterogeneous and multifaceted. Nat Rev Immunol. 22(3):173–187. doi: 10.1038/s41577-021-00571-6.
13.0Fridlender, Z.G., Sun, J., Kim, S. Kapoor, V., Cheng, G., Ling, L. et al. (2009). Polarization of tumor-associated neutrophil phenotype by TGF-β: “N1” versus “N2” TAN. Cancer Cell. 16(3):183–194. doi: 10.1016/j.ccr.2009.06.017.
14. Brandau, S., Dumitru, C.A., Lang, S. (2013). Protumor and antitumor functions of neutrophil granulocytes. Semin Immunopathol. 35(2):163–176. doi: 10.1007/s00281-012-0344-6.
15. Antuamwine, B.B., Bosnjakovic, R., Hofmann-Vega, F. Wang, X., Theodosiou, T. et al. (2023). N1 versus N2 and PMN-MDSC: a critical appraisal of current concepts on tumor-associated neutrophils and new directions for human oncology. Immunol Rev. 314(1):250–279. doi: 10.1111/imr.13176.
16. Uehara, K., Nakanishi, Y., Shimoda, T. Taniguchi, H., Akasu, T., Moriya, Y. (2007). Clinicopathological significance of microscopic abscess formation at the invasive margin of advanced low rectal cancer. Br J Surg. 94(2):239–243. doi: 10.1002/bjs.5575.
17. Berry, R.S., Xiong, M.J., Greenbaum, A., Mortaji, P., Nofchissey, R.A., Schultz, F. et al. (2017). High levels of tumor-associated neutrophils are associated with improved overall survival in patients with stage II colorectal cancer. PLoS One. 12(12):e0188799. doi: 10.1371/journal.pone.0188799.
18. Galdiero, M.R., Bianchi, P., Grizzi, F., Di Caro, G., Basso, G., Ponzetta, A. et al. (2016). Occurrence and significance of tumor-associated neutrophils in patients with colorectal cancer. Int J Cancer. 139(2):446–456. doi: 10.1002/ijc.30076.
19. Sachs, U.J., Andrei-Selmer, C.L., Maniar, A. Weiss, T., Paddock, C., Orlova, V.V. et al. (2007). The neutrophil-specific antigen CD177 is a counter-receptor for platelet endothelial cell adhesion molecule-1 (CD31). J Biol Chem. 282(32):23603–23612. doi: 10.1074/jbc.M701120200.
20. Ducker, T.P., Skubitz, K.M. (1992). Subcellular localization of CD66, CD67, and NCA in human neutrophils. J Leukoc Biol. 52(1):11–16. doi: 10.1002/jlb.52.1.11.
21. Väyrynen, J.P., Vornanen, J.O., Sajanti, S., Böhm J.P., Tuomisto A., Mäkinen, M.J. (2012). An improved image analysis method for cell counting lends credibility to the prognostic significance of T cells in colorectal cancer. Virchows Arch. 460(5):455–465. doi: 10.1007/s00428-012-1232-0.
22. Caruso, R.A., Bellocco, R., Pagano, M., Bertoli, G., Rigoli, L., Inferrera, C. (2002). Prognostic value of intratumoral neutrophils in advanced gastric carcinoma in a high-risk area in northern Italy. Mod Pathol. 15(8):831–837. doi: 10.1097/01.MP.0000020391.98998.6B.
23. Ieni, A., Branca, G., Parisi, A., Fedele, F., Irato, E., Venuti, A., Caruso, R.A. (2015). Neutrophil-rich gastric carcinoma in the integrated cancer registry of eastern Sicily, Italy. Anticancer Res.

35(1):487–492.
24. Millar, E.K., Beretov, J., Sarris, M., Lee, C.S. (2001). Mucinous differentiation in colonic adenocarcinoma is associated with a reduction in tumour-infiltrating lymphocytes. Eur J Surg Oncol. 27(3):273–277. doi: 10.1053/ejso.2000.1094.
25. Elomaa, H., Tarkiainen, V., Äijälä, V.K., Sirniö, P., Ahtiainen, M., Sirkiä, O. et al. (2025). Associations of mucinous differentiation and mucin expression with immune cell infiltration and prognosis in colorectal adenocarcinoma. Br J Cancer. 132(7):660–669. doi: 10.1038/s41416-025-02960-3.
26. Friedl, P., Alexander, S. (2011). Cancer invasion and the microenvironment: plasticity and reciprocity. Cell. 147(5):992–1009. doi: 10.1016/j.cell.2011.11.016.
27. Ueno, H., Kajiwara, Y., Shimazaki, H., Shinto, E., Hashiguchi, Y., Nakanishi, K. et al. (2012). New criteria for histologic grading of colorectal cancer. Am J Surg Pathol. 36(2):193–201. doi: 10.1097/PAS.0b013e318235edee.
28. Lugli, A., Kirsch, R., Ajioka, Y., Bosman, F., Cathomas, G., Dawson, H. et al. (2017). Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol. 30(9):1299–1311. doi: 10.1038/modpathol.2017.46.
29. Haddad, T.S., Lugli, A., Aherne, S., Barresi, V., Terris, B., Bokhorst, J.M. et al. (2021). Improving tumor budding reporting in colorectal cancer: a Delphi consensus study. Virchows Arch. 479(3):459–469. doi: 10.1007/s00428-021-03059-9.
30 Barresi, V., Branca, G., Ieni, A. Reggiani Bonetti, L., Baron, L., Mondello, S., Tuccari, G. (2014). Poorly differentiated clusters (PDCs) as a novel histological predictor of nodal metastases in pT1 colorectal cancer. Virchows Arch. 464(6):655–662. doi: 10.1007/s00428-014-1580-z.
31. Barresi, V., Reggiani Bonetti, L., Ieni, A., Caruso, R.A., Tuccari, G. (2015). Histological grading in colorectal cancer: new insights and perspectives. Histol Histopathol. 30(9):1059–1067. doi: 10.14670/HH-11-633.
32. Barresi, V., Reggiani Bonetti, L., Ieni, A., Caruso, R.A., Tuccari, G. (2017). Poorly differentiated clusters: clinical impact in colorectal cancer. Clin Colorectal Cancer. 16(1):9–15. doi: 10.1016/j.clcc.2016.06.002.
33. Haddad, T.S., Bokhorst, J.M., van den Dobbelsteen, L., Öztürk, S.K., Baumann, E., van Vliet, S. et al. (2025). Tumor budding and poorly differentiated clusters as a biological continuum in colorectal cancer invasion and prognosis. Sci Rep. 15(1):16944. doi: 10.1038/s41598-025-00866-x.
34. Haddad, T.S., van den Dobbelsteen, L., Öztürk, S.K., Geene, R., Nijman, I.J., Verrijp, K. et al. (2023). Pseudobudding: ruptured glands do not represent true tumor buds. J Pathol. 261(1):19–27. doi: 10.1002/path.6146.
35. Caruso, R.A., Speciale, G., Inferrera, C. (1994). Neutrophil interaction with tumour cells in small early gastric cancer: ultrastructural observations. Histol Histopathol. 9(2):295–303.
36. Caruso, R.A., Bonanno, A., Finocchiaro, G., Cavaliere, R., Gitto, G., Plutino, F.M. et al. (2009). Ultrastructural observations on inflammatory angiogenesis in gastric carcinomas with massive neutrophil infiltration. Ultrastruct Pathol. 33(1):1–5. doi: 10.1080/01913120802636696.
37. Caruso, R.A., Rigoli, L., Parisi, A., Fedele, F., Bonanno, A., Paparo, D. et al. (2013). Neutrophil-rich gastric carcinomas: light and electron microscopic study of 9 cases with particular reference to neutrophil apoptosis. Ultrastruct Pathol. 37(3):164–170. doi: 10.3109/01913123.2013.768746.
38. Caruso, R.A., Fedele, F., Rigoli, L. Branca, G., Bonanno, A., Quattrocchi, E. et al. (2013). Apoptotic-like tumor cells and apoptotic neutrophils in mitochondrion-rich gastric adenocarcinomas: a comparative study with light and electron microscopy between these two forms of cell death. Rare Tumors. 5(2):68–71. doi: 10.4081/rt.2013.e18.
39. Siriaunkgul, S., Tavassoli, F.A. (1993). Invasive micropapillary carcinoma of the breast. Mod Pathol. 6(6):660–662.
40. Li, S., Gao, S., Qin, L. Ding, C., Qu, J., Cui, Y. et al. (2025). Micropapillary structure: a natural tumor collective invasion model with enhanced stem-like properties. Cancer Sci. 116(2):308–321. doi: 10.1111/cas.16396.
41. Luna-Moré, S., Gonzalez, B., Acedo, C., Rodrigo, I., Luna, C. (1994). Invasive micropapillary carcinoma of the breast. A new special type of invasive mammary carcinoma. Pathol Res Pract. 190(7):668–674. doi: 10.1016/S0344-0338(11)80745-4.
42. Caruso, R.A., Cicciarello, R., Gagliardi, M.E., Albiero, F., Costa, G., Fedele, F. et al. (2008).

Micropapillary carcinoma of the breast with necrosis-like cell death: a case report. Ultrastruct Pathol. 32(4):153–159. doi: 10.1080/01913120802179473.
43. Barresi, V., Branca, G., Vitarelli, E., Tuccari, G. (2014). Micropapillary pattern and poorly differentiated clusters represent the same biological phenomenon in colorectal cancer: a proposal for a change in terminology. Am J Clin Pathol. 142(3):375–383. doi: 10.1309/AJCPFEA7KA0SBBNA.
44. Hong, M., Kim, J.W., Shin, M.K., Kim, B.C. (2017). Poorly differentiated clusters in colorectal adenocarcinomas share biological similarities with micropapillary patterns as well as tumor buds. J Korean Med Sci. 32(10):1595–1602. doi: 10.3346/jkms.2017.32.10.1595.
45. Shivji, S., Conner, J.R., Barresi, V., Kirsch, R. (2020). Poorly differentiated clusters in colorectal cancer: a current review and implications for future practice. Histopathology. 77(3):351–368. doi: 10.1111/his.14128.
46. Ono, Y., Yilmaz, O. (2024). Emerging and under-recognised patterns of colorectal carcinoma morphologies: a comprehensive review. J Clin Pathol. 77(7):439–451. doi: 10.1136/jcp-2023-208816.
47. Khayyata, S., Basturk, O., Adsay, N.V. (2005). Invasive micropapillary carcinomas of the ampullo-pancreatobiliary region and their association with tumor-infiltrating neutrophils. Mod Pathol. 18(11):1504–1511. doi: 10.1038/modpathol.3800460.
48. Reid, M.D., Basturk, O., Thirabanjasak, D., Hruban, R.H., Klimstra, D.S., Bagci, P. et al. (2011). Tumor-infiltrating neutrophils in pancreatic neoplasia. Mod Pathol. 24(12):1612–1619. doi: 10.1038/modpathol.2011.113.
49. Ryota, H., Ishida, M., Ebisu, Y., Yanagimoto, H., Yamamoto, T., Kosaka, H. et al. (2021). Clinicopathological characteristics of pancreatic ductal adenocarcinoma with invasive micropapillary carcinoma component with emphasis on the usefulness of PKCζ immunostaining for detection of reverse polarity. Oncol Lett. 22(1):525. doi: 10.3892/ol.2021.12786.
50. Wen, P., Xu, Y., Frankel, W.L., Shen, R. (2008). Invasive micropapillary carcinoma of the sigmoid colon: distinct morphology and aggressive behavior. Int J Clin Exp Pathol. 1(5):457–460.
51. Arai, K., Shimazaki, K., Takahashi, K., Hazama, H., Ohata, K., Sonoda, A. et al. (2025). Relevance of immunohistochemistry for tumorigenic tumor-infiltrating neutrophils and reverse polarity in colonic micropapillary adenocarcinoma: a case report. Case Rep Pathol. 2025:9365437. doi: 10.1155/crip/9365437.
52. Zhang, Q., Ming, J., Zhang, S., Li, B., Yin, L., Qiu, X. (2015). Micropapillary component in gastric adenocarcinoma: an aggressive variant associated with poor prognosis. Gastric Cancer. 18(1):93–99. doi: 10.1007/s10120-014-0350-6.
53. Barresi, V., Branca, G., Ieni, A., Rigoli, L., Tuccari, G., Caruso, R.A. (2015). Phagocytosis (cannibalism) of apoptotic neutrophils by tumor cells in gastric micropapillary carcinomas. World J Gastroenterol. 21(18):5548–5554. doi: 10.3748/wjg.v21.i18.5548.
54. Caruso, R.A., Muda, A.O., Bersiga, A., Rigoli, L., Inferrera, C. (2002). Morphological evidence of neutrophil-tumor cell phagocytosis (cannibalism) in human gastric adenocarcinomas. Ultrastruct Pathol. 26(5):315–321. doi: 10.1080/01913120290104593.
55. Caruso, R.A., Fedele, F., Finocchiaro, G., Arena, G., Venuti, A. (2012). Neutrophil-tumor cell phagocytosis (cannibalism) in human tumors: an update and literature review. Exp Oncol. 34(3):306–311.
56. Lu, T., Li, W. (2025). Neutrophil engulfment in cancer: friend or foe? Cancers (Basel). 17(3):384. doi: 10.3390/cancers17030384.
57. Brown, G.C. (2024). Cell death by phagocytosis. Nat Rev Immunol. 24(2):91–102. doi: 10.1038/s41577-023-00921-6.
58. Humble, J.G., Jayne, W.H., Pulvertaft, R.J. (1956). Biological interaction between lymphocytes and other cells. Br J Haematol. 2:283–294. doi: 10.1111/j.1365-2141.1956.tb06700.x.
59. Gupta, N., Jadhav, K., Shah, V. (2017). Emperipolesis, entosis and cell cannibalism: demystifying the cloud. J Oral Maxillofac Pathol. 21(1):92–98. doi: 10.4103/0973-029X.203763.
60. Overholtzer, M., Mailleux, A.A., Mouneimne, G., Normand, G., Schnitt, S.J., King, R.W. et al. (2007). A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion. Cell. 131(5):966–979. doi: 10.1016/j.cell.2007.10.040.
61. Steiner, P., Wiesbauer, L., Kerschbaum, H.H., Zierler, S. (2026). Non-apoptotic programmed cell death: from ultrastructural characterization to emerging therapeutic opportunities. Cells. 15(2):111. doi: 10.3390/cells15020111.
62. Malorni, W., Matarrese, P., Tinari, A., Farrace, M.G., Piacentini, M. (2007). Xeno-cannibalism: a

survival “escamotage”. Autophagy. 3(1):75–77. doi: 10.4161/auto.3439.
63. Matarrese, P., Ciarlo, L., Tinari, A., Piacentini, M., Malorni, W. (2008). Xeno-cannibalism as an exacerbation of self-cannibalism: a possible fruitful survival strategy for cancer cells. Curr Pharm Des. 14(3):245–252. doi: 10.2174/138161208783413239.
64. Kulshrestha, R., Negi, A., Bhutani, I., Saxena, H., Rani, M., Menon, B. et al. (2023). Tumor cell phagocytosis (cannibalism) in lung cancer: possible biomarker for tumor immune escape and prognosis. Am J Transl Res. 15(3):1935–1940.
65. Druzhkova, I., Ignatova, N., Shirmanova, M. (2023). Cell-in-cell structures in gastrointestinal tumors: biological relevance and clinical applications. J Pers Med. 13(7):1149. doi: 10.3390/jpm13071149.
66. Templeton, A.J., McNamara, M.G., Šeruga, B., Vera-Badillo, F.E., Aneja, P., Ocaña, A. et al. (2014). Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 106(6):dju124. doi: 10.1093/jnci/dju124.
67. Cupp, M.A., Cariolou, M., Tzoulaki, I., Aune, D., Evangelou, E., Berlanga-Taylor, A.J. (2020). Neutrophil-to-lymphocyte ratio and cancer prognosis: an umbrella review of systematic reviews and meta-analyses of observational studies. BMC Med. 18(1):360. doi: 10.1186/s12916-020-01817-1.
68. Koc, D.C., Mănescu, I.B., Mănescu, M., Dobreanu, M. (2024). A review of the prognostic significance of neutrophil-to-lymphocyte ratio in nonhematologic malignancies. Diagnostics (Basel). 14(18):2057. doi: 10.3390/diagnostics14182057.
69. Buonacera, A., Stancanelli, B., Colaci, M., Malatino, L. (2022). Neutrophil-to-lymphocyte ratio: an emerging marker of the relationships between the immune system and diseases. Int J Mol Sci. 23(7):3636. doi: 10.3390/ijms23073636.
70. Howard, R., Scheiner, A., Kanetsky, P.A., Egan, K.M. (2019). Sociodemographic and lifestyle factors associated with the neutrophil-to-lymphocyte ratio. Ann Epidemiol. 38:11–21.e6. doi: 10.1016/j.annepidem.2019.07.015.
71. Angkananard, T., Anothaisintawee, T., McEvoy, M., Attia, J., Thakkinstian, A. (2018). Neutrophil–lymphocyte ratio and cardiovascular disease risk: a systematic review and meta-analysis. Biomed Res Int. 2018:2703518. doi: 10.1155/2018/2703518.
72. Clausen, F., Behrens, H.M., Krüger, S., Röcken, C. (2020). Sexual dimorphism in gastric cancer: tumor-associated neutrophils predict patient outcome only for women. J Cancer Res Clin Oncol. 146(1):53–66. doi: 10.1007/s00432-019-03082-z.
73. Quaas, A., Pamuk, A., Klein, S., Attia, J., Thakkinstian, A., Barutcu, A.G. et al. (2021). Sex-specific prognostic effect of CD66b-positive tumor-infiltrating neutrophils (TANs) in gastric and esophageal adenocarcinoma. Gastric Cancer. 24(6):1213–1226. doi: 10.1007/s10120-021-01197-2.
74. Davidson, W.M., Smith, D.R. (1954). A morphological sex difference in the polymorphonuclear neutrophil leucocytes. Br Med J. 2(4878):6–7. doi: 10.1136/bmj.2.4878.6.
75. Richter, M., Maier-Begandt, D., Jablonska, J., Silvestre-Roig, C. (2025). Sex differences in neutrophil biology. J Leukoc Biol. 117(12):qiaf161. doi: 10.1093/jleuko/qiaf161.
76. Hoffmann, J.P., Liu, J.A., Seddu, K., Klein, S.L. (2023). Sex hormone signaling and regulation of immune function. Immunity. 56(11):2472–2491. doi: 10.1016/j.immuni.2023.10.008.
77. Sciarra, F., Campolo, F., Franceschini, E., Carlomagno, F., Venneri, M.A. (2023). Gender-specific impact of sex hormones on the immune system. Int J Mol Sci. 24(7):6302. doi: 10.3390/ijms24076302.
78. Huang, X., Pan, Y., Ma, J., Kang, Z., Xu, X., Zhu, Y. et al. (2018). Prognostic significance of the infiltration of CD163+ macrophages combined with CD66b+ neutrophils in gastric cancer. Cancer Med. 7(5):1731–1741. doi: 10.1002/cam4.1420.
79. Qin, Y., Liu, Y., Dong, P., Zou, W-B., Li, Z., Huang L. (2025). The heterogeneous roles of neutrophils in gastric cancer: scaffold or target? Cell Mol Biol Lett. 30:71. doi: 10.1186/s11658-025-00744-4.
80. Abe, H., Morikawa, T., Saito, R., Yamashita, H., Seto, Y., Fukayama, M. (2016). In Epstein–Barr virus-associated gastric carcinoma a high density of CD66b-positive tumor-associated neutrophils is associated with intestinal-type histology and low frequency of lymph node metastasis. Virchows Arch. 468(5):539–548. doi: 10.1007/s00428-016-1915-z.
81. Zhao, J.J., Pan, K., Wang, W., Chen, J.G., Wu, Y.H., Lu. L. et al. (2012). The prognostic value of tumor-infiltrating neutrophils in gastric adenocarcinoma after resection. PLoS One. 7(3):e33655. doi: 10.1371/journal.pone.0033655.
82. Zhang, H., Liu, H., Shen, Z., Lin, C., Wang, X., Qin, J. et al. (2018). Tumor-infiltrating neutrophils

is prognostic and predictive for postoperative adjuvant chemotherapy benefit in patients with gastric cancer. Ann Surg. 267(2):311–318. doi: 10.1097/SLA.0000000000002058.
83. Wahnou, H., El Kebbaj, R., Hba, S., Ouadghiri, Z., El Faqer, O., Pinon, A. et al. (2025). Neutrophils and neutrophil-based drug delivery systems in anti-cancer therapy. Cancers (Basel). 17(7):1232. doi: 10.3390/cancers17071232.
84. Xue, J., Zhao, Z., Zhang, L., Xue, L., Shen, S., Wen, Y. et al. (2017). Neutrophil-mediated anticancer drug delivery for suppression of postoperative malignant glioma recurrence. Nat Nanotechnol. 12(7):692–700. doi: 10.1038/nnano.2017.54.
85. Ju, C., Wen, Y., Zhang, L., Wang, Q., Xue, L,, Shen, J., Zhang, C. (2019). Neoadjuvant chemotherapy based on Abraxane/human neutrophils cytopharmaceuticals with radiotherapy for gastric cancer. Small. 15:e1804191. doi: 10.1002/smll.201804191.
86. Li, Y., Han, Y., Su, R., Liu, Y., Chong, G., Xu, D. et al. (2020). Photosensitizer-laden neutrophils are controlled remotely for cancer immunotherapy. Cell Rep. 33(11):108499. doi: 10.1016/j.celrep.2020.108499.
87. Chang, Y., Cai, X., Syahirah, R., Yao, Y., Xu, Y., Jin, G. et al. (2023). CAR-neutrophil-mediated delivery of tumor-microenvironment-responsive nanodrugs for glioblastoma chemo-immunotherapy. Nat Commun. 14(1):2266. doi: 10.1038/s41467-023-37872-4.

Received May 21, 2026, accepted May 22, 2026, published online June 22, 2026