Clinicopathological significance of gastric poorly differentiated medullary carcinoma

(低分化髄様胃癌の臨床病理学的特徴)

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Running title

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Abstract

Poorly differentiated gastric adenocarcinoma of solid type is known to show a

clinicopathological diversity, but its morphological characteristics have rarely been

investigated. In this study, we defined poorly differentiated medullary carcinoma indicating

the following three characteristics: (i) more than 90% of the entire tumor were composed of

poorly differentiated adenocarcinoma in a medullary growth, (ii) the tumor exhibited an

expansive growth at the tumor margin, and (iii) special types such as an

α-fetoprotein-producing carcinoma, neuroendocrine carcinoma, and carcinoma with

lymphoid stroma were excluded. Based on the definition, we subclassified the poorly

differentiated gastric adenocarcinoma of solid type into the two groups: medullary carcinoma

and non-medullary carcinoma, and clinicopathologically analyzed 23 cases of medullary

carcinomas and 38 cases of non-medullary carcinomas. The medullary carcinomas less

frequently displayed lymphatic invasion, venous invasion, and lymph node metastasis,

compared with the non-medullary carcinoma ($P < 0.001$, $P = 0.002$, and $P < 0.001$,

respectively). The patients with medullary carcinomas significantly showed better

disease-free survival ($P = 0.017$). This is the first study to demonstrate that poorly

differentiated adenocarcinoma of solid type can be subclassified into tumors with low and

high malignant potentials. Gastric poorly differentiated medullary carcinoma is considered to

be a novel histological type predicting good patients' prognosis.

There are various histopathological classifications of gastric cancer. The Japanese

classification of gastric carcinoma classifies it into the differentiated and undifferentiated

types (11, 19), while the Lauren classification categorizes gastric carcinomas into the

intestinal and diffuse types (15), which mostly correspond with differentiated and

undifferentiated types, respectively. However, gastric carcinomas often consist of a mixture

of various histological patterns. According to the Japanese and Lauren classifications, the

most quantitatively superior histological pattern became the diagnostic category

(histopathological diagnosis), but the other histological patterns are not reflected in the

diagnostic category. Histological types according to the World Health Organization (WHO)

classification indicates tubular/papillary/mucinous adenocarcinoma, and poorly cohesive

carcinoma (5). The WHO classification recommends that the diagnostic category includes not

only the most quantitatively superior histological pattern, but also the other histological

patterns.

According to the Japanese classification of gastric carcinoma, poorly differentiated

carcinomas are divided into two groups, *i.e.*, solid type and non-solid type. The solid type

exhibits a sheet-like solid growth pattern with scanty stroma, whereas the non-solid type

shows a small alveolar, cord-like, or isolated pattern with abundant fibrous stroma. The

clinicopathological features of the poorly differentiated carcinoma of solid type less

frequently exhibit lymphatic invasion and lymph node metastasis, and show better patients'

prognosis compared to the non-solid type carcinoma (9, 14, 21, 25). However, poorly

differentiated carcinomas of solid type are thought to be a heterogenous histological group,

because the Japanese classification defines the histological type of gastric carcinomas as a

quantitatively superior histological pattern. In this study, therefore, we have defined

histological criteria of "poorly differentiated medullary carcinoma" as a carcinoma of pure

(homogenous) poorly differentiated carcinoma of solid type without the other histological

patterns. First of all, we devided the poorly differentiated carcinoma of solid type into the two

groups, *i.e.* medullary carcinoma and non-medullary carcinoma. The medullary carcinoma

was composed of homogenous poorly differentiated carcinoma in a medullary growth pattern.

Moreover, we evaluated the clinicopathological characteristics and prognosis for medullary

carcinoma and non-medullary carcinoma.

Materials and methods

Patients

This study evaluated 61 consecutive surgical cases of poorly differentiated gastric

adenocarcinoma of solid type treated between January 2005 and December 2014 at the

Hirosaki University Hospital after obtaining each patients' informed consent to use their

clinical records and pathological specimens. The case series comprised 39 males and 22

females with a median age of 74 years (range, 59–94 years). The carcinomas were located in

the lower third (26 cases), middle third (26 cases), and upper third (9 cases) stomach

according to the anatomic location (11). Curative resection and regional lymph node

dissection were dependent on the location of primary tumors. Distal gastrectomy was

performed for 35 patients, and total gastrectomy was performed for 26 patients. Borrmann

classification type 1 or 2 was observed in 31 cases and type 3, 4, or 5 was seen in 30 cases (5).

The mean tumor diameter was 66.2 mm. Survival data were obtained from hospital medical

records. The median observation period for 35 cases was 36.5 months.

Pathological analysis

All surgically resected specimens were fixed using 10% formalin, embedded in paraffin, and

stained using hematoxylin and eosin (H&E) for pathological evaluation. Gastric carcinomas

were evaluated according to the Japanese classification of gastric carcinoma (11) and staged

using the TNM classification of the International Union Against Cancer (UICC) (23). The

histological features assessed in the largest cross-sectional tumor section were as follows:

histological type, depth of invasion (T-grade), lymph node metastasis, lymphatic invasion,

venous invasion, and infiltration pattern of tumor (INF). The degree of lymphatic and venous

invasion was classified as follows: 0, no invasion; 1, mild invasion; 2, moderate invasion; and

3, severe invasion. INF was categorized into three groups: INFa, cancer nests showing

expansive growth and presenting the clear borderline between tumor tissue and stroma; INFb,

intermediate patterns of growth between INFa and INFc; and INFc, scirrhous growth with an

unclear border at the invasive front. We defined a medullary carcinoma indicating the

following three characteristics: (i) more than 90% of the entire tumor were composed of

poorly differentiated adenocarcinoma of solid type; (ii) the tumor exhibited an expansive

growth at the tumor margin, *i.e.* INFa; and (iii) the special types such as an α-fetoprotein

(AFP)-producing carcinoma, neuroendocrine carcinoma, and carcinoma with lymphoid

stroma were excluded. Based on the definition, we subclassified the poorly differentiated

gastric adenocarcinoma of solid type into the two groups: medullary carcinoma and

non-medullary carcinoma.

Immunohistochemistry

Immunohistochemical examination was performed on deparaffinized sections using the

standard avidin-biotin-peroxidase complex method with automated immunostainer

(Benchmark XT; Ventana Medical System, Tucson, AZ, USA). Podoplanin (D2-40) was used

for revealing lymphatic endothelium and clarifying lymphatic invasion. The antibody we

used was D2-40 (1:100, clone D2-40; Dako, Glostrup, Denmark).

Statistical analysis

The Pearson's chi-square test was used to assess potential associations between categorical

variables with the use of adjusted residual analysis. The Kaplan‑Meier method was used to

construct survival curves and differences in survival were evaluated using the log‑rank test.

Differences were considered to be statistically significant if the *P* value was <0.05. Statistical

evaluations were performed using EZR (Saitama Medical Center, Jichi Medical University,

Saitama, Japan) (12) and PASW statistics softwares (version 18.0; SPSS, Inc., Chicago, IL,

USA).

Results

Gross and histological findings

Review of 61 cases of poorly differentiated adenocarcinoma of solid type identified 23

medullary carcinomas and 38 non-medullary carcinomas. Macroscopically, the most

medullary carcinomas were expansively ulcerated tumors with sharply demarcated and raised

margins, *i.e.*, type 2 tumor according to the Bormann classification (Fig. 1A). Cross-sectional

tumor segments showed expansive growth with clear margins (Fig. 1B). The most

non-medullary carcinomas were infiltratively ulcerated tumors lacking definite margins, *i.e.*,

type 3 tumor according to the Bormann classification (Figs. 1C, D). Microscopically,

medullary carcinomas showed expansive growth with few glandular structures and a distinct

border demarcating the surrounding tissue, *i.e.*, INFa (Fig. 2A). The medullary carcinomas

showed a solid pattern (Fig. 2B). Medullary carcinomas usually had either no or mild

lymphatic invasion and moderate to severe venous invasion (Figs. 3A, B). Non-medullary

carcinomas showed not only a solid carcinoma component, but also the other histological

components such as poorly differentiated adenocarcinoma of non-solid type (Figs. 2C, D).

The non-medullary carcinomas usually exhibited moderate to severe lymphatic and venous

invasion. Immunohistochemical staining for D2-40 revealed lymphatic endothelium, and

clarified lymphatic invasion (Figs. 3C, D). The histological components of medullary and

non-medullary carcinomas are summarized in Table 1. More than half (52.2%, 12/23) of

medullary carcinomas were composed of poorly differentiated adenocarcinoma of solid type,

only. On the other hand, the majority (92.1%, 35/38) of the non-medullary carcinomas were

composed of the plural histological components.

Clinicopathological findings

The clinicopathological findings pertaining to medullary carcinoma and non-medullary

carcinoma are summarized in Table 2. There were significant differences in Borrmann

classification, lymph node metastasis, lymphatic invasion, venous invasion, and INF between

medullary carcinoma and non-medullary carcinoma ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P =$

0.002, and $P < 0.001$, respectively). In comparison with the non-medullary carcinoma, the

medullary carcinomas less frequently showed lymph node metastasis, lymphatic invasion,

and venous invasion. There was no significant difference in the depth of invasion ($P = 0.335$).

Detailed results of lymphatic and venous invasion are summarized in Table 3. In

comparison with the non-medullary carcinoma, the medullary carcinoma less frequently

showed lymphatic invasion (ly0, 1 & v0, 1 and ly0, 1 & v2, 3 in Table 3). In comparison with

medullary carcinoma, non-medullary carcinoma more frequently exhibited venous invasion

(ly2, 3 & v2, 3 in Table 3).

Patient's survival rates

There were significant differences in disease-free survival between the patients with

medullary carcinoma and those with non-medullary carcinoma (Fig. 4A, $P = 0.017$), but there

was no significant difference in overall survival between these groups (Fig. 4B, $P = 0.079$).

Discussion

We defined a medullary carcinoma indicating the following three characteristics: (i) more than 90% of the entire tumor were composed of poorly differentiated adenocarcinoma of solid type; (ii) the tumor exhibited an expansive growth at the tumor margin, *i.e.* INFa; and (iii) special types were excluded. Based on the definition, we subclassified the poorly differentiated gastric adenocarcinoma of solid type, into the two groups: medullary carcinoma and non-medullary carcinoma. Medullary carcinomas showed significantly reduced lymphatic invasion, venous invasion, and lymph node metastasis, resulting in a better prognosis compared to non-medullary carcinoma.

There are a few reports on the subclassification of poorly differentiated adenocarcinoma of solid type (1, 25). Adachi *et al.* divided it into 2 groups: pure poorly differentiated medullary carcinoma, in which a solid pattern occupied more than 80% of the tumor area, and mixed poorly differentiated medullary carcinoma, in which a solid pattern occupied 50%–80% of the tumor area (1). Compared to mixed poorly differentiated medullary carcinoma, pure poorly differentiated medullary carcinoma showed expansive growth and inflammatory infiltration. However, the pure poorly differentiated medullary carcinoma did not indicate low frequency of lymphatic invasion/venous invasion/lymph node

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metastasis, and there was no statistical significance in patients' outcomes between the two

groups. Their "pure poorly differentiated medullary carcinoma" is similar to our category, but

they did not mention the infiltration pattern at the tumor margin indicating INF, and exclusion

of the special types of gastric carcinoma. Song *et al.* has reported that INFa of the gastric

carcinoma was associated with good patients' prognosis (24). INF has been shown to be a

useful prognostic factor not only for gastric carcinoma, but also for colorectal, gallbladder,

and urothelial carcinomas (8, 18, 20). We excluded the special types of gastric carcinoma

such as AFP-producing carcinoma, neuroendocrine carcinoma, and carcinoma with lymphoid

stroma. AFP-producing carcinomas are known to be high-grade malignancy with frequent

liver metastasis, and show a poor patients' prognosis (10, 13). Neuroendocrine carcinomas

also exhibit a poor patients' prognosis (17, 22). In contrast, carcinomas with lymphoid stroma

are distinct entities with better patients' prognosis (26, 27). In the present study, we defined

medullary carcinoma with three histological characteristics, and assumed that it showed

clearly distinct clinicopathological features, compared to the non-medullary carcinomas.

Recent studies have demonstrated that microsatellite instability represented a

hypermutable phenotype caused by a DNA mismatch repair deficiency, one of the initiating

pathways for gastric cancer (4, 6, 16). The microsatellite instability in gastric tumors was

associated with the histological types such as poorly differentiated adenocarcinoma of solid

type, and papillary adenocarcinoma, typically indicating expansive growth (2, 7). The poorly

differentiated gastric adenocarcinoma with microsatellite instability is considered to be a

counterpart of colorectal medullary carcinoma, which is representative of colorectal

carcinomas with microsatellite instability (3). In the near future, we will analyze any

relationships between the microsatellite instability and the gastric medullary carcinoma that

we have proposed in this study.

In conclusion, we should classify the poorly differentiated gastric adenocarcinoma of

solid type, into the two distinct groups: medullary carcinoma and non-medullary carcinoma.

The gastric poorly differentiated medullary carcinoma is a novel histological type predicting

good patients' prognosis.

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Tables

Table 1. Histological components of medullary carcinoma and non-medullary carcinoma

Histological subclassification[†]	Medullary carcinoma (n = 23)	Non-medullary carcinoma (n = 38)	*P*-value
por (solid), only	12 (52.2%)[*]	3 (7.9%)	
por (solid)>por (non-solid)	1 (4.4%)	12 (31.6%)[*]	
por (solid)>well, mod	9 (39.0%)	18 (47.4%)	
por (solid)>muc, sig	1 (4.4%)	5 (13.2%)	<0.001

[†]Histological subclassification according to the Japanese classification system for gastric carcinomas: por (solid), poorly differentiated adenocarcinoma of solid type; por (non-solid), poorly differentiated adenocarcinoma of non-solid type; well, well differentiated tubular adenocarcinoma; mod, moderately differentiated tubular adenocarcinoma; muc, mucinous adenocarcinoma; sig, signet-ring cell carcinoma.

[*]Statistically significant association by adjusted residual analysis (*P* < 0.05)

Table 2. Clinicopathological characteristics of medullary carcinoma and non-medullary carcinoma

	Poorly differentiated adenocarcinoma of solid type (n = 61)		
	Medullary carcinoma (n = 23)	non-medullary carcinoma (n = 38)	P-value
Age (years)			0.777
≥70	15	27	
<70	8	11	
Gender			0.137
Male	12	27	
Female	11	11	
Gastric location			0.523
Lower	11	15	
Middle or upper	12	23	
Borrmann classification			<0.001
Type 1 or 2	22	9	
Type 3, 4, or 5	1	29	
Tumor diameter (mm)			0.902
<50	7	11	
≥50	16	27	
Depth of invasion[†]			0.335
T2 or T3	15	20	
T4	8	18	
Lymph node metastasis			<0.001
pN (+)	6	31	
Lymphatic invasion[‡]			<0.001
ly0 or ly1	16	4	
ly2 or ly3	7	34	
Venous invasion[‡]			0.002
v0 or v1	9	2	
v2 or v3	14	36	
INF[‡]			<0.001
a	23	3	
b or c	0	35	

[†]Depth of invasion according to TNM classification

[‡]Lymphatic invasion, venous invasion, and INF according to the Japanese classification of gastric carcinoma

Table 3. Lymphatic and venous invasion of medullary carcinoma and non-medullary carcinoma

ly[†] & v[‡]	Medullary carcinoma (n = 23)	Non-medullary carcinoma (n = 38)	P-value
ly0, 1 & v0, 1	5 (21.7%)[*]	0 (0%)	
ly0, 1 & v2, 3	11 (47.8%)[*]	4 (10.5%)	
ly2, 3 & v0, 1	4 (17.4%)	2 (5.3%)	
ly2, 3 & v2, 3	3 (13.0%)	32 (84.2%)[*]	<0.001

[†]ly, lymphatic invasion according to Japanese classification of gastric carcinoma: 0, no invasion; 1, mild invasion; 2, moderate invasion; and 3, severe invasion.

[‡]v, venous invasion according to Japanese classification of gastric carcinoma: 0, no invasion; 1, mild invasion; 2, moderate invasion; and 3, severe invasion.

[*]Statistically significant association by adjusted residual analysis ($P < 0.05$)

Figure legends

Figure 1. Gross findings of medullary carcinoma (**A, B**) and non-medullary carcinoma (**C, D**). (**A**) Medullary carcinomas were ulcerated tumors with sharply demarcated and raised margins. (**B**) A tumor cross-section of medullary carcinoma showed expansive growth with a clear margin. (**C**) Non-medullary carcinoma comprised an ulcerated tumor without a definite border. (**D**) A tumor cross-section of non-medullary carcinoma showed an unclear margin.

Figure 2. Histological findings of medullary carcinoma (**A, B**) and non-medullary carcinoma (**C, D**). (**A**) Medullary carcinoma showed a fungating tumor with expansive growth, *i.e.*, type 2 tumor according to the Borrmann classification and a distinct border with the surrounding tissue, *i.e.*, INFa. (**B**) Medullary carcinoma indicated only a solid pattern with few glandular structures. (**C**) Non-medullary carcinoma showed an ulcerated tumor with infiltrating growth, *i.e.* type 3 tumor according to the Borrmann classification. (**D**) A non-solid pattern in non-medullary carcinoma indicated a small alveolar and isolated pattern with abundant stroma.

Figure 3. Vascular invasion of medullary carcinoma and non-medullary carcinoma. (**A, B**)

Medullary carcinomas usually display moderate to severe venous invasion (**A**, H&E

staining; **B**, Elastica van Gieson staining). (**C, D**) Non-medullary carcinomas usually showed

not only moderate to severe venous invasion but also lymphatic invasion.

Immunohistochemical staining for D2-40 revealed the lymphatic endothelium and clarified

lymphatic invasion (**C**, H&E staining; **D**, immunohistochemistry for D2-40).

Figure 4. Patients' prognosis using Kaplan-Meier survival curves. (**A**) Patients with

medullary carcinoma and non-medullary carcinoma had significantly different disease-free

survival ($P = 0.017$). (**B**) There was no significant difference in overall survival between

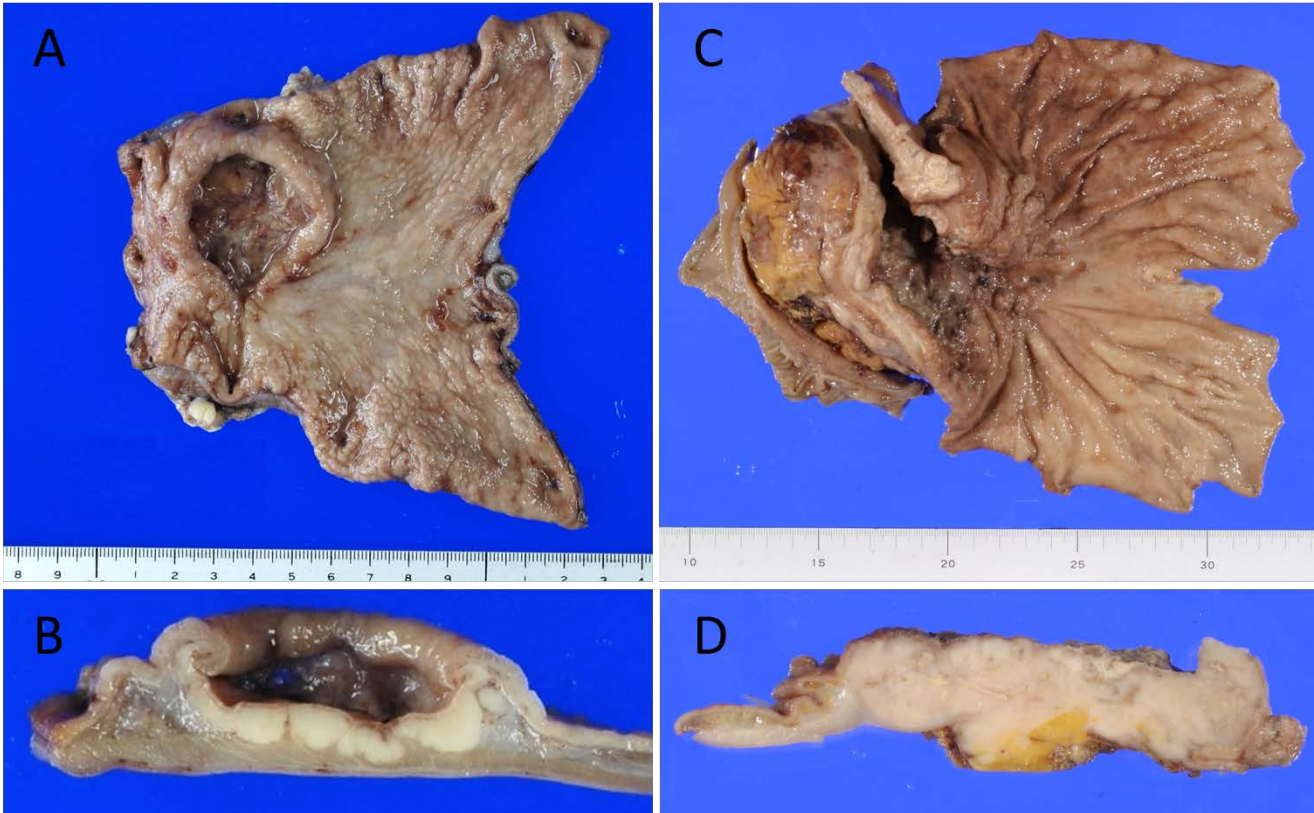patients with medullary carcinoma and non-medullary carcinoma ($P = 0.079$).
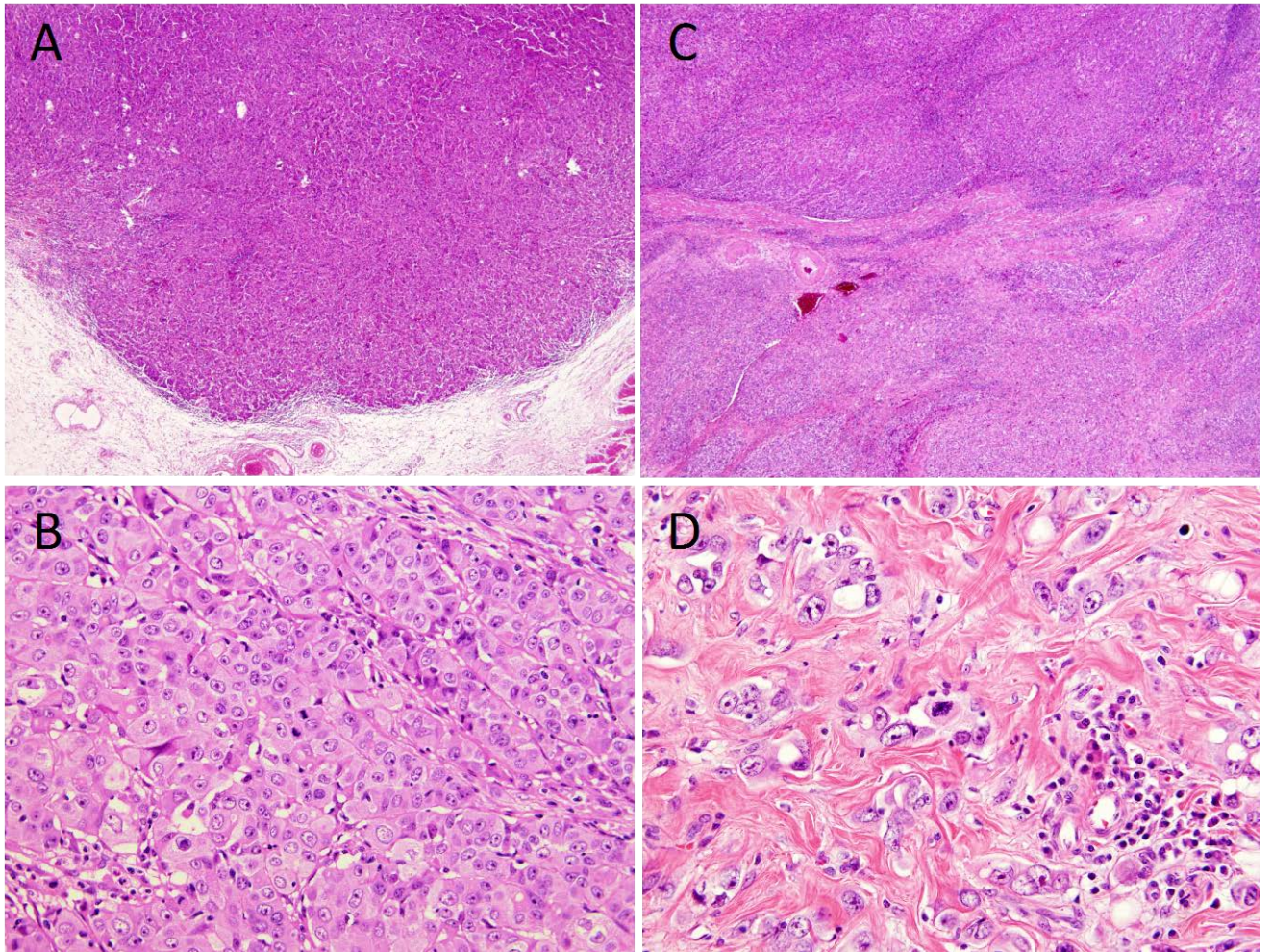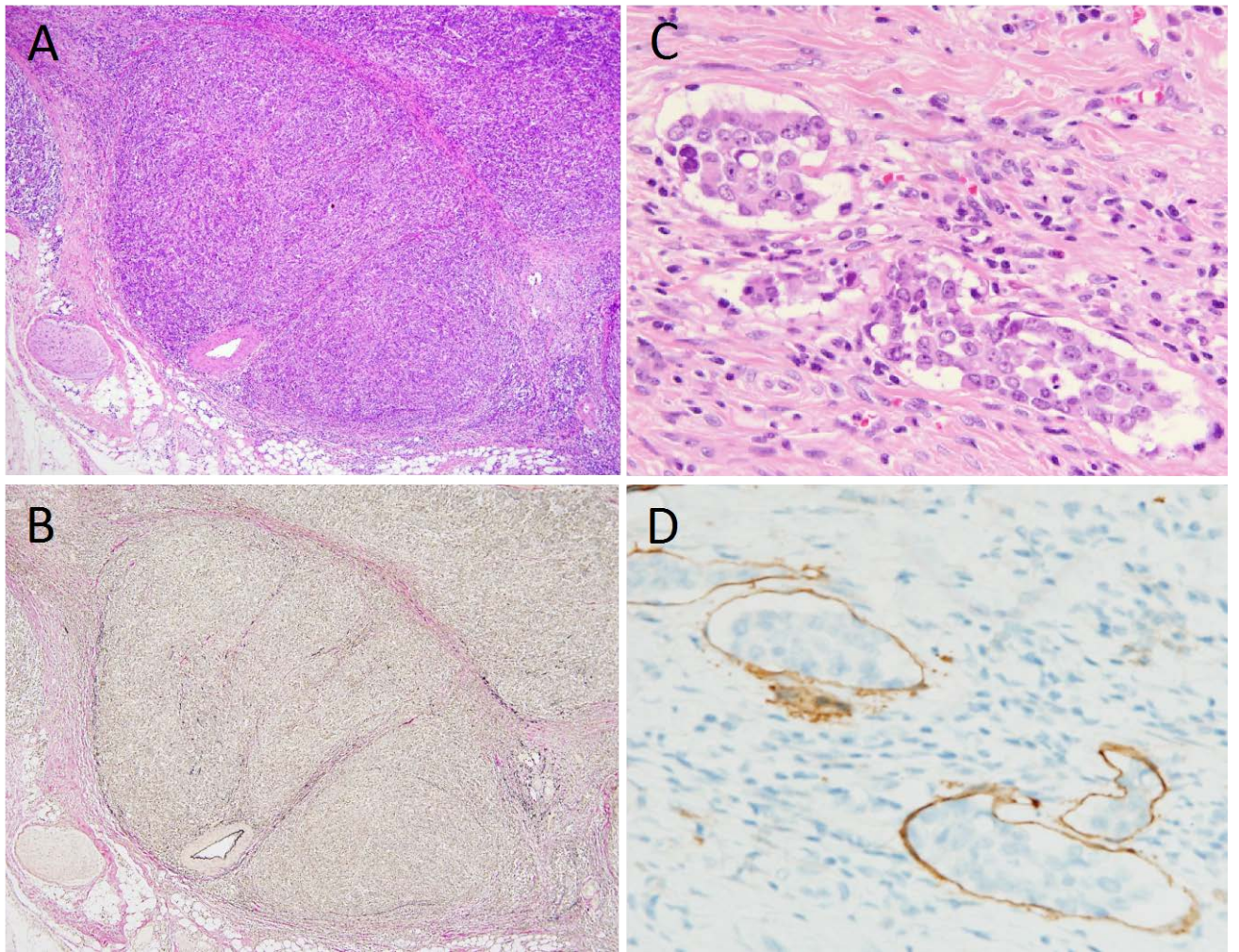
Figure 1

Figure 2

Figure 3

Figure 4

(A)

P=0.017

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(B)

P=0.079

: medullary carcinoma
: non-medullary carcinoma

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