Abstract. Aim: The association of motilin, ghrelin, leptin, gastrin, pepsinogen (PG) I and II with cancer chemotherapy-associated dyspepsia syndrome (CADS) was investigated in 35 patients with breast cancer receiving first cycle of 5-fluorouracil, cyclophosphamide, epirubicin (FEC60) chemotherapy. Patients and Methods: The onset of dyspeptic symptoms on days 3 and 10 after chemotherapy identified patients with and without CADS. Gastrointestinal symptoms were scored with the Gastrointestinal Symptom Scoring Rate (GSRS) questionnaire. Gastrointestinal peptides were evaluated by enzyme–linked immunosorbent assay. Results: Twenty-one patients (60%) had CADS. The area under the curve (AUC) of ghrelin was higher, whereas that of PGI, PGII and motilin were lower in patients with CADS compared to those without. In patients with CADS, the AUC of PGI and PGII negatively correlated with the GSRS indigestion cluster. Conclusion: Impairment of gastrointestinal motility suggested by low motilin concentrations and mucosal damage mirrored by an increase of ghrelin seem to be involved in the onset of CADS in patients during chemotherapy for breast cancer.

In order to prevent breast cancer-related recurrence and death after primary definitive treatment, adjuvant therapy is usually given, often with a regimen such as 5-fluorouracil (5FU), cyclophosphamide, and epirubicin (1).

Despite prophylactic administration of anti-emetics, many patients undergoing chemotherapy still experience different gastrointestinal (GI) symptoms such as early satiety, anorexia, nausea and vomiting. All of these symptoms have been collectively described as cancer chemotherapy-associated dyspepsia syndrome (CADS) and can be complications of the disease itself and/or the treatment (2).

To date, the prophylactic use of 5-hydroxytryptamine 3 (5-HT3) receptor antagonists such as ondansetron, is well-established in patients with nausea and vomiting undergoing chemotherapy. In contrast to other antiemetics, ondansetron is effective and well-tolerated, and is able to improve GI symptoms linked to motility disorders (e.g. irritable bowel syndrome, diarrhea, carcinoid syndrome) (3).

In view of the concept of integrated bi-directional signalling between the gut and brain, a number of GI peptides (e.g. motilin, ghrelin, and leptin) have been indicated as having a pathophysiological role in functional dyspepsia (FD) and recent findings suggest that they may also be associated with the onset of CADS (4). Among them, the 22-amino-acid peptide motilin, secreted by endocrine M cells in the mucosa of the proximal small intestine, participates in controlling the pattern of smooth muscle contractions in the upper GI tract, in particular with the interdigestive migrating motor complex (MMC). A study performed on patients with ovarian cancer undergoing chemotherapy showed a transient decrease of motilin after drug administration in those who experienced nausea and vomiting (5).

The likely co-secretion of intestinal motilin and ghrelin suggests the concerted actions of these two hormones on GI motility (6). Ghrelin, a 28-amino-acid peptide, exhibits central and peripheral activities in the regulation of food intake and energy balance (7), as well as in relation to FD (8). Ghrelin has been studied in both human and animal models. Administration of this peptide has been shown to
reduce the symptoms of CADS induced in rats with cisplatin (9), whereas clinical studies have reported that plasma ghrelin, as well as feeding activity and nutritional status, significantly decreased in patients with esophageal cancer treated with cisplatin-based chemotherapy (10).

Another crucial peptide involved in regulating appetite and metabolism is leptin (11). Leptin receptors are present in each of the major components of the central nervous system feeding centers. Through these receptors, leptin affects feeding behaviour (12). Due to its peculiar functions, leptin may have a protective role in the upper gut during states of injury (13), and higher serum leptin concentrations have been found in patients with dysmotility-like dyspepsia (14). Gastrin and pepsinogens I (PGI) and II (PGII), pro-enzymes of pepsin, are also related to dyspeptic symptoms, being tightly related to chronic conditions such as Helicobacter pylori infection, atrophic gastritis and gastric cancer (15-17).

To our knowledge, few data are available on the possible involvement of these GI peptides in the onset of chemotherapy-related GI side-effects, such as CADS. The majority of available reports derives from studies performed on laboratory animals treated with potent antineoplastic drugs such as cisplatin (9). However, feeding activity and reactions to chemotherapeutic agents greatly differ between humans and rodents, and new drugs have fewer side-effects compared to cisplatin. Therefore, there is a need to investigate for effects of novel protocols on these peptides in patients with extra GI cancer in relation to symptoms beyond nausea and vomiting.

In this framework, the aim of the study was to evaluate CADS symptoms and their association with the serum GI peptide levels in patients treated with a non–cisplatin–based chemotherapy protocol for breast cancer. Thus, peptides known to be useful biomarkers for the detection of GI inflammatory and motility involvement, namely motilin, ghrelin, leptin and gastrin, along with PGI and PGII, were measured and correlated with the Gastrointestinal Symptom Scoring Rate (GSRS) questionnaire (18) during the first cycle of adjuvant FEC60 chemotherapy.

Patients and Methods

Patients. This prospective observational study focused on consecutive patients with breast cancer who underwent adjuvant chemotherapy. Patients were recruited from the Oncology Unit of the Saverio De Bellis National Institute of Digestive Diseases, and the Medical Oncology Unit of the Giovanni Paolo II National Cancer Institute. They were screened by two investigators (GR and FG) and recruited according to the inclusion criteria and their willingness to participate. The study began in July 2010 and enrolment of patients ended in September 2011. Fifty female patients with breast cancer who underwent adjuvant chemotherapy were enrolled.

The study was performed in accordance with the Helsinki Declaration and all participants gave written informed consent to participation before enrolment. The protocol was approved by the local Scientific and Ethics Committees (227/DSC2011) and the study was registered at www.clinicaltrials.gov, registration number: NCT01382667.

The eligibility criteria for the study were the following: i) a histopathological diagnosis of infiltrating ductal carcinoma; ii) stage II or III cancer according to the criteria of the International Union against Cancer (UICC); iii) the ability to take in soft solids orally; iv) Eastern Cooperative Oncology Group performance status (PS) within the range of 0 to 1.5; v) adequate function of major organs; vi) no other active malignancy. Pre-treatment clinical staging was based on liver echography; computed tomography scans of the neck, chest, and the upper and lower abdomen as continuous 5-mm-thick sliced total body bone scan.

The exclusion criteria were: hypertension, diabetes mellitus and other pathologies (e.g. systemic, endocrine, and collagen-related diseases). Participants had not taken antibiotics, probiotics, vitamins, minerals, non-steroidal anti-inflammatory or prokinetic drugs, bismuth, antacids, H2-receptor antagonists, omeprazole, sucralfate or misoprostol in the four weeks prior to the study and had no previous history of dyspeptic symptoms, gastric or duodenal ulcers or major GI surgery.

To complete the clinical characteristics of the patients, the Mini Nutritional Assessment (MNA) and the Cumulative Index Rating Scale (CIRS) were administered before the start of the adjuvant chemotherapy. The MNA test consists of multi-dimensional measurements (anthropometric, global assessment, dietary questionnaire, and subjective assessment) that allow researchers to identify patients with: i) adequate nutritional status, score ≥24; ii) protein-caloric malnutrition, score <17; iii) risk of malnutrition, score=17-23.5 (19). The CIRS is a measure of multimorbidity (20). Outcome measures were the illness severity score (CIRS-IS, mean of all single item scores) and the comorbidity index (CIRS-CI, number of single items with a score of 3, 4 or 5). The final score of the CIRS is the sum of the 14 individual items scoring theoretically from 0 to 56. However, a very high score is impossible because it is not compatible with life (21).

Adjuvant chemotherapy regimen. After surgical resection of the tumor and lymph nodes (primary therapy), all enrolled patients received adjuvant chemotherapy. The adjuvant regimen consisted of epirubicin-based chemotherapy. Specifically, the chemotherapy regimen consisted of 5FU at 600 mg/m², epirubicin at 60 mg/m² and cyclophosphamide at 600 mg/m² (FEC60) administered every 21 days for six cycles.

There was no significant difference in the background for tumour status and systemic condition between patients who received adjuvant chemotherapy. Supportive therapy and prophylaxis against expected side-effects were provided. All of the patients were pre-medicated with intravenous ondansetron (4 mg), infused one hour prior to the administration of the scheduled drugs. Hypersensitivity reactions were prophylactically treated with intravenous dexamethasone (8 mg), which was infused one hour prior to the administration of chemotherapy. Granulocyte-stimulating factor (G-CSF) was used for febrile neutropenia when deemed necessary.

Symptom assessment. In order to categorize patients into groups with and without CADS, the participants were asked about dyspepsia in terms of their subjective judgment at the intermediate visits (performed on day 3 and 10 after the beginning of the first cycle of adjuvant chemotherapy) by answering the question: “Did
you have GI symptoms of dyspepsia after the start of the treatment?” The possible answers were “Yes” or “No”. If the answer was “Yes” they were then asked: “Was dyspepsia intense enough to require medication?” Only patients who answered “No” continued the study in order to avoid bias on the GI peptide profiles induced by drugs (i.e. proton pump inhibitor, and prokinetic drugs) apart from the scheduled FEC60 regimen.

Patients were evaluated for their GI symptoms with the GRSRS, a validated questionnaire for dyspeptic symptoms (18) before and at the end of the first cycle of chemotherapy. The GRSRS utilizes a seven-level Likert scale (1 to 7), depending on intensity and frequency of GI symptoms experienced during the previous weeks. A higher score indicates more symptoms of discomfort. Combination scores among the questions can determine the following five clusters: “reflux syndrome” (halitosis, heartburn, dysphagia and acid regurgitation: max. score=28); “abdominal pain” (stomach and colic ache, gastric hunger pains, and nausea: max. score=42); “indigestion syndrome” (postprandial fullness, early satiety, borborygmus, bloating, burping, and increased flatus: max. score=42); “diarrhea syndrome” (increased frequency of evacuation, loose stools, and urgent need to defecate: max. score=21); and “constipation syndrome” (reduced frequency of evacuation, hard stools, and feeling of incomplete evacuation: max. score=21). The maximal achievable score was 154.

Gastrointestinal peptides. Peripheral venous blood was collected in vacutainer tubes in the morning (at 08.00 h) after fasting from midnight and before the first cycle of chemotherapy (day 0), as well as on days 3, 10, 14 and 21 after the beginning of treatment. The clotted samples were centrifuged at 1,600 × g for 15 min and the separated sera were stored at –80˚C until assayed. The serum levels of motilin, total ghrelin and leptin were assayed by Human Motilin Elisa kit (Cusabio Biotech Co, Wuhan, P.R.China), Human Total Ghrelin Elisa kit (Ray Biotech Inc, Norcross, GA, USA), and Human Leptin ELISA kit (Diagnostic Biochem Canada Inc. London ON, Canada), respectively. Serum gastrin, PGI, PGII were evaluated by respective ELISA kits of Biohit Oyj (Helsinki, Finland).

Statistical analysis. Data are expressed as the mean±SE, unless otherwise specified. To avoid violation of the assumption of normal distribution, non-parametric tests were performed. GI peptide evaluations were performed as raw values at five time-points and the area under the curve with respect to ground (AUCg). The AUCg allows researchers to assess if any change occurred over time, and is indicated in the case of a profile in which data in a given data point allows researchers to assess if any change occurred over time, and is indicated in the case of a profile in which data in a given data point are lower than the basal value. The AUCg was calculated using the trapezoid formula described by Pruessner (22). All data were evaluated by Mann-Whitney rank sum test. The relationship between parameters was investigated by Spearman correlation analysis. Statistical significance was set at p<0.05. The software package used for the statistical analysis was StataCorp 2005 (Stata Statistical Software: Release 9; College Station, TX, USA).

Results

Patients’ characteristics. Table I lists anthropometric and clinical characteristics of the patients. Thirty-five females completed the study (aged 59.2±9.7 years). At the start of the study, the mean patient body-mass index (BMI) was 29.5±5.9 kg/m² and the MNA was 25.4±2.8, in the range of adequate nutrition state. Performance status was generally fair with

<table>
<thead>
<tr>
<th>Number of patients</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>59.2±9.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.5±5.9</td>
</tr>
<tr>
<td>Stage</td>
<td>40% Stage II; 60% Stage III</td>
</tr>
<tr>
<td>Multidimensional evaluations</td>
<td></td>
</tr>
<tr>
<td>GRSRS (=22, absence of symptoms)</td>
<td>25.7±5.1</td>
</tr>
<tr>
<td>CIRS-IS (max=5)</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>CIRS-CI (max=13)</td>
<td>0.4±0.8</td>
</tr>
<tr>
<td>MNA (≥24, adequate nutrition state)</td>
<td>25.4±2.8</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.6±3.2</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.3±0.1</td>
</tr>
<tr>
<td>Lymphocytes (/µl)</td>
<td>1534.4±63.9</td>
</tr>
<tr>
<td>Serum iron (µg/dl)</td>
<td>54.6±3.2</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>107.7±3.3</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.84±0.1</td>
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</table>

Data are expressed as the mean±SD. GRSRS: Gastrointestinal symptom scoring rate; CIRS-IS: cumulative index rating scale–illness severity; CIRS-CI: cumulative index rating scale–comorbidity index; MNA: mini nutritional assessment; BMI: body mass index.

Eastern Cooperative Oncology Group=0-2. Clinically, 40% of patients were at stage II and 60% were at stage III.

Table I also summarizes the results of laboratory tests at the start of the study. Laboratory parameters were in the normal range.

Sixty percent (n=21) of patients developed CADS, whilst 40% (n.14 patients) did not. Table II shows the GRSRS cluster pattern recorded before and after the first chemotherapy cycle in the two groups. The two groups exhibited a similar pattern in all the GRSRS clusters at baseline. At the end of the first FEC60 cycle, patients with CADS reported a slightly but significantly higher score, by 23.2%, in the “indigestion syndrome”, compared to those without CADS (9.5±0.7 vs. 7.3±0.5, respectively, p=0.03, Mann-Whitney rank sum test). The most frequent symptom of the “indigestion syndrome” was burping (61.9% of patients with CADS vs. 38.5% without). In order of frequency, the other symptoms were: increased flatus (42.8% vs. 23.1%), postprandial fullness (33.3% vs. 7.7%), bloating (28.6% vs. 15.4%), and early satiety (23.8% vs. 7.7%). No difference was found in the other GRSRS clusters between the two groups.

GI peptide concentrations in patients with and without CADS. Figure 1 plots the serial changes in the GI peptide concentrations in the patients. Comparing each recording day between those with CADS and those without, in patients with CADS, motilin concentrations were 30.2% lower on day 3, 60.1% on day 10, (p=0.04) and 41.4% on day 14, (p=0.04), and 48.3% on day 21, (p=0.02) (Figure 1, A).
In patients with CADS, ghrelin concentrations were 18.0% higher on day 3, 10.2% on day 10, 7.9% on day 14 and 12.8% on day 21 than in those without, but not significantly (Figure 1, B).

For leptin and gastrin levels, no significant differences were found between the two groups (Figure 1, C and D). On each recording day, PGI and PGII concentrations were approximately 15-20% lower in patients with CADS compared to those without, but not reaching statistical significance (Figure 1, E and F). In contrast, the PGI/PGII ratio was quite similar in both groups of patients (data not shown).

In order to evaluate the total release of GI peptides over the time of observation, the AUCg of each peptide was analyzed according to the presence or absence of CADS. The AUCg of motilin (Figure 1, A) was significantly lower in patients with CADS compared to those without (89.2±21.9 vs. 140.4±29.6; p=0.03), whilst the AUCg of ghrelin (Figure 1, B) was significantly higher in patients with CADS than in those without (108.2±10.2 vs. 77.8±8.8, respectively; p=0.04).

The AUCg of leptin (Figure 1, C) was lower, without reaching statistical significance, in patients with CADS (60.2±10.4 vs. 75.8±11.6, respectively).

The AUCg of gastrin (Figure 1, D) did not differ between groups (16.3±4.8 vs. 13.6±4.0). Finally, the AUCg of PGI and PGII (Figure 1, E and F, respectively) decreased significantly in patients with CADS compared to those without (AUCg of PGI: 232.2±27.0 vs. 351.6±39.8, p=0.04; AUCg of PGII: 31.6±3.8 vs. 46.6±6.6, p=0.04).

**Correlation between GI peptide concentrations and GSRS symptom clusters.** In the patients with CADS, the “indigestion syndrome” cluster score negatively and significantly correlated only with the AUCg of PGI ($r_s=-0.69$, $p=0.002$), and PGII ($r_s=-0.57$, $p=0.007$) (Spearman correlation analysis) (Figure 2). In contrast, in the patients without CADS, no correlation was found between the “indigestion syndrome” cluster score and the AUCg of the investigated peptides (data not shown).

**Discussion**

The FEC60 regimen is the most commonly used FEC regimen as adjuvant treatment for patients with breast cancer. It is generally well-tolerated even if stomatitis, dysphagia, diarrhea (in association or not with nausea) or dyspepsia are present during its administration (2).

After the prophylactic administration of ondansetron, a selective 5-HT3 receptor antagonist proven to be effective in treatment of nausea and vomiting during the first 24 h of chemotherapy (23), many patients undergoing FEC60 therapy may suffer from other different GI symptoms, thus experiencing a poor quality of life along with increased health costs (24). In our study, more than half of the patients experienced symptoms similar to those in dysmotility-like FD recorded in the GSRS questionnaire as “indigestion syndrome” cluster (burping, postprandial fullness, early satiety, borborygmus, bloating, eructation, and increased flatus: max. score=42), “diarrhoea syndrome” (increased frequency of evacuation, loose stools, and urgent need to defecate: max. score=21), and “constipation syndrome” (reduced frequency of evacuation, hard stools, and feeling of incomplete evacuation: max. score=21). Maximal achievable score was 154.

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**Table II. Gastrointestinal symptom scoring rate total and cluster scores in patients with (n=21) and those without (n=14) CADS.**

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 21</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>With</td>
<td>Without</td>
</tr>
<tr>
<td>Total GSRS score</td>
<td>25.4±1.6</td>
<td>24.6±1.2</td>
</tr>
<tr>
<td>Reflux syndrome</td>
<td>4.5±0.2</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>6.4±0.4</td>
<td>6.4±0.3</td>
</tr>
<tr>
<td>Indigestion syndrome</td>
<td>7.6±0.6</td>
<td>7.2±0.5</td>
</tr>
<tr>
<td>Diarrhoea syndrome</td>
<td>2.9±0.2</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td>Constipation syndrome</td>
<td>4.7±0.8</td>
<td>3.2±0.5</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±SEM. Mann-Whitney rank sum test. Clusters are: “reflux syndrome” (halitosis, heartburn, dysphagia and acid regurgitation: max. score=28), “abdominal pain” (stomach ache epigastric, colic etc.), gastric hunger pains, and nausea: max. score=42), “indigestion syndrome” (postprandial fullness, early satiety, borborygmus, bloating, eructation, and increased flatus: max. score=42), “diarrhoea syndrome” (increased frequency of evacuation, loose stools, and urgent need to defecate: max. score=21), and “constipation syndrome” (reduced frequency of evacuation, hard stools, and feeling of incomplete evacuation: max. score=21). Maximal achievable score was 154.
In the current study, comparing each time point, motilin was the only peptide found at significantly lower concentrations, as well as lower total release, in patients with CADS compared to those without. This finding could explain the dysmotility-like symptoms mirrored by the significant increase of the GSRS “indigestion syndrome” cluster score. The present findings are in agreement with existing data in literature. Previously, Hursti et al. observed a transient decrease in circulating motilin after chemotherapy in patients with ovarian cancer who had chemotherapy-related nausea and vomiting (5). The authors suggested that inhibition of GI motility can be important for the development of GI symptoms and decreased plasma levels of motilin after chemotherapy may play a role in the pathogenesis of CADS.

For what ghrelin is concerned, its concentrations increased over the time of observation in both groups, with a significant higher AUCg in patients with CADS than those without. Various studies have evaluated the association of total plasma ghrelin and FD, although with opposite results (27, 28). Recently, Hiura et al. found that cisplatin-based chemotherapy significantly reduced total plasma ghrelin in humans (10), while studies in animal models with cancer...
chemotherapy-induced dyspepsia showed both increased plasma levels of ghrelin (26) and an improvement of symptoms after administration of this peptide (9). Our results agree with these latter findings and allow us to hypothesize that higher levels of this peptide, acting as prokinetic (29), could be a defensive response to toxic challenges of chemotherapy in attempt to correct impaired motility in the upper GI tract.

Leptin has also been linked to states of injury to the gut (13) and dysmotility-like dyspepsia (14), however, no differences were found between the two groups in terms of circulating peptide levels or AUCg. The evaluation of circulating leptin levels may not have been sensitive enough to reveal any modification since changes in leptin content might mostly be present in the gastric mucosa as already demonstrated in patients with gastritis (30).

As far as gastrin is concerned, the first cycle of FEC60 induced an increase in its circulating levels in both groups, without significant differences between them. This finding is in agreement with data reporting a protective role of gastrin on GI mucosal integrity in response to different toxic stimuli, as already demonstrated in rats treated with ethanol or acidified aspirin (31). PGI and PGII progressively increased in a parallel manner in the two patients groups, but the AUCg of both pepsinogens was lower in patients with CADS than in those without. Moreover, the AUCg of PGI and PGII negatively-correlated with the GSRS “indigestion syndrome” cluster score. This evidence is intriguing since it is in accordance with findings of lower PGI levels in patients with non-ulcer dyspepsia compared to healthy controls (32). On the other hand, it is in disagreement with a more recent study from Tahara et al. who found that higher PGI levels were significantly associated with a greater number of dyspeptic symptoms, at least in H. pylori-positive patients (33). Differences in the patient enrolment, as well as in the aetiology of dyspeptic disorders, may account for discrepancies among these reports. To our knowledge the present study is the first report on the circulating pepsinogen levels during chemotherapy and in relation to the onset of CADS. There is thus a need to study larger populations, more representative of these patients, in order to corroborate the present data.

Supported by reports in literature, our results suggest the evaluation of GI peptides as possible tools for tackling dyspeptic disorders after chemotherapy. Our findings underscore the fact that various pathophysiological mechanisms seem to be involved in CADS onset and the characterization of a different role of markers of inflammation such as ghrelin and pepsinogens, linked to a large decrease in motilin concentration, could help to understand the causes of this syndrome.

In the present study, some considerations need to be taken into account when evaluating our findings. The present, was an observational study and only patients with mild dyspeptic symptoms were enrolled, consequently the GSRS score was not very high, albeit the presence of mild symptoms allowed us to avoid administration of drugs known to affect GI motility and/or peptide patterns. In addition, the only prophylactic use of ondansetron before the start of chemotherapy eliminated the potential interference of ondansetron in the GI peptide profile, it being able to inhibit ghrelin-induced changes in gut motility (34), produce constipation and modulate fatigue and depression (35).

In conclusion, the concept of the gut forming the centre of an integrated gut–brain–energy axis, modulating appetite, metabolism and digestion, also opens up new paradigms in the treatment of GI disorders in patients with cancer. The hormones that modulate gastric motility and modify eating behaviour may be targeted to develop drugs that reduce dyspeptic symptoms. The gut–brain axis may, therefore, provide a range of therapeutic opportunities that allow deliverers of a more holistic treatment of upper GI disorders (4, 13, 36).

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References


