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**Comparison of diagnostic tools for Sjögren' s syndrome, with emphasis on Sialography, Histopathology, and Ultrasonography**

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## **ABSTRACT.**

**Objective.** The present study examined the reliability and correlation of sialography, salivary gland biopsy, and ultrasonography for Sjögren' s syndrome (SS), and evaluated the usefulness of ultrasonography as a diagnostic tool for SS in comparison with sialography and histopathology.

**Methods.** Seventy-three patients who underwent sialography, ultrasonography, and salivary gland biopsy were included in this study. The study evaluated the diagnostic reliability and correlation of each kind of examination with SS.

**Results.** There was a statistically significant difference in the sensitivities of sialography and histopathology, in the specificities of sialography and ultrasonography, and in the accuracies of sialography and both of ultrasonography and histopathology. The correlation coefficient ( $r$ ) between sialography and ultrasonography was significantly higher than the others and indicated a good correlation.

**Conclusions.** Ultrasonography can be used as a diagnostic tool for SS with its advantage of non-invasiveness and facility.

## **INTRODUCTION**

The Sjögren' s syndrome (SS) is an autoimmune disease involving the exocrine glands as main target organs. The criteria for a diagnosis of SS have been controversial. In Japan, the diagnostic criteria for SS were revised in 1999 by the Research Group for Sjögren' s syndrome of the Japanese Society for the Promotion of Science<sup>1</sup>. These criteria are based on the diagnostic criteria for Sjögren' s syndrome established by the European Community Study Group and consists of 4 items : 1) histopathology of biopsy specimens either from the labial salivary glands or lachrymal glands; 2) an oral examination (a) sialography, or (b) combination of sialometry (chewing gum test or Saxon test) and salivary scintigraphy; 3) an ocular examination (a) combination of the Schirmer test and the rose bengal test, or (b) combination of the Schirmer test and fluoescetin staining test; 4) the serologic test of anti-Ro/SS-A antibody or anti-La/SS-B antibody<sup>1,2</sup>. The diagnosis of SS can be made when the patients meet two or more of the above 4 items. The oldest imaging procedure, sialography, has maintained its position as the method of choice for exploring the ductal system of the salivary glands because of its high diagnostic reliability<sup>3</sup>. Since the 1990s, CT, MRI, MR sialography, and ultrasonography have also been applied to diagnose SS<sup>4-8</sup>. Among these, ultrasonography is the most convenient and economical examination which furthermore is noninvasive. Although the criteria for SS in ultrasonography have been

established by a few researchers, they have not been applied generally and not included among a global diagnostic examination for SS<sup>6-9</sup>. It has also been reported that the sensitivity of diagnosis of SS by ultrasonography ranged from 40% to 100%, and that it is not necessarily superior to other methods of examination in diagnostic reliability<sup>6,7</sup>. This study investigated the diagnostic reliability and correlation between a diagnosis employing sialography, ultrasonography, and a histopathological diagnosis, and evaluated the usefulness of ultrasonography as a diagnostic tool for SS.

## **MATERIALS AND METHODS**

From April 2001 through April 2007, 244 patients who visited the Department of Oral and Maxillofacial Surgery and Dental Radiology, Hokkaido University Hospital constituted the patient population. All of the patients had undergone ocular examinations and serologic test for Sjögren's syndrome at the Division of Rheumatology, Department of Internal Medicine or the Department of Ophthalmology. The patients were referred to the Department of Oral and Maxillofacial Surgery and Dental Radiology for oral examinations. The patients studied in this study consisted of 73 patients (4 male and 69 female) aged 13–68 years (mean age 48 years), all of whom underwent 3 oral examinations: sialography, salivary gland biopsy, and ultrasonography after obtaining the patients' informed consent. Additionally, salivary secretion test (chewing gum test or Saxon test) was also carried out in some of the patients for diagnostic work-up purposes. Among 73 patients, 36 had been diagnosed as SS by ocular examination and serologic test, while remaining 37 had been diagnosed as non-SS but complained of sicca symptom. The diagnosis of SS on sialography and histopathology was made based on the revised Japanese criteria for Sjögren's syndrome. (Table. 1)

**Sialography.** Cannulation was performed by a 20G catheter (Therflow/ Terumo, ATOM Medical, Tokyo, Japan) into an orifice of the parotid main duct with the help of a fine silver wire. The catheter was ligatured to the buccal mucosa under local anesthesia to prevent the catheter falling off and contrast fluids leaking. An automatic injector (Truth/ Tokyo, Japan) was used to inject 2 ml of 76% diatrizoate sodium (Urografin/Schering, Osaka, Japan) into Stensen's duct at the rate of 0.0125 ml/sec. Serial lateral images were obtained continuously during and after the injection to observe the ductal, acino-parenchymal, and functional phases. After removal of the ligature, patients were advised to stimulate salivary gland secretion, massaging the glands and imbibing citric flavored liquid to enhance washout of remaining contrast

fluid. Two dental radiologists (30 and 10 years experience) evaluated the sialograms and performed the diagnosis based on the classifications of Rubin and Holt<sup>10</sup>. (Table. 2, Fig. 1) Stage 1 or higher were diagnosed as positive, but when the peripheral ductal dilation was observed, it was assessed as suspicious (possible) positive. Where the diagnosis of the radiologists differed, discussion was made and a diagnosis was agreed on.

**Ultrasonography.** Sonographic examinations were performed using HDI3000 (ATL, Washington, USA). Bilateral parotid and submandibular glands were scanned in the axial and coronal planes. B-mode multifoci images were taken with the center frequency of 5 to 12 MHz. Patients were scanned in supine with the necks extended and the heads turned a little toward the opposite side. Sonographic evaluations were performed independently by dental radiologists with 25 years experience who were not informed of the sialographic diagnosis. Sonographic diagnosis was performed based on the inhomogeneity of the parenchyma of the glands established by Salaffi, et al<sup>9</sup>. Grade 3 or higher were diagnosed as SS, and grade 1-2 were assessed as suspicious (possible) positive. (Table. 3, Fig. 2)

**Biopsy.** Labial salivary gland biopsy was performed under local anesthesia by oral surgeons. A lower lip mucosal incision was made between the midline and the commissure and at least three labial gland samples were obtained. The histopathological findings were graded based on Greenspan<sup>11</sup> classification by experienced oral pathologists. Grade 3 or higher were diagnosed as SS and grade 2 was assessed as suspicious (possible) positive. (Table. 4, Fig. 3)

**Statistical analysis.** Statistical analyses were performed by the chi-square test, and a P value of less than 0.05 was considered to be statistically significant. We performed all statistical analyses with SPSS Statistic Base 17.0 (SPSS Japan Inc, Tokyo, Japan).

## RESULTS

The sensitivity of sialography was 83.3% and that of ultrasonography and histopathology were 77.8% and 63.9%, respectively. There was a statistically significant difference between the sialography and histopathology results ( $p < 0.05$ ). The specificity of sialography was 94.4% and that of ultrasonography and histopathology was 78.8% and 91.4%, respectively. There was a statistically significant difference between the sialography and ultrasonography ( $p < 0.05$ ). The accuracy of the sialography was 89.0%, and that of the ultrasonography and histopathology were both 78.1%. There were statistically significant differences

between the sialography and both the ultrasonography and histopathology ( $p < 0.05$ ). This result showed that sialography was the most reliable diagnostic tool for SS (Table. 5). The incidences of hyposalivation (Saxon or chewing gum test) was 77.8% in SS patients and 54.3% in non-SS patients, respectively. Correlation corresponding to negative, suspicious, positive SS was evaluated and calculations between the diagnostic tools showed that sialography-ultrasonography had the highest correlation ( $r = 0.58$ ) and those of sialography-histopathology, ultrasonography-histopathology were 0.35 and 0.50, respectively (Table. 6). Complications resulting from the examinations were observed in 4 patients. Two patients developed acute sialadenitis due to the sialography procedure, and this was overcome with the administration of antibiotics. Two patients complained of persistent pain of the lip where the probe was inserted, but the pain disappeared in a few weeks and no neuroparalysis remained. No complications related to the ultrasonographic examination were noted.

## DISCUSSION

The reliability of the sialography based diagnosis in the study here was comparable to that reported in a large institutional analysis by Fujibayashi, et al<sup>13</sup>. There the sensitivity, specificity, and accuracy of sialography according to the revised Japanese criteria, was reported as 89.1%, 91.4%, and 89.9%, respectively. The diagnostic reliability of the ultrasonography in our study was equivalent to alternative oral examination results, salivary secretion tests and salivary scintigraphy, as reported by Fujibayashi<sup>13</sup>. In that study, sensitivity, specificity, and accuracy were 75.7%, 78.7%, and 76.9%, respectively<sup>11</sup>. Salaffi, et al<sup>14</sup>. compared ultrasonography of salivary glands in primary Sjögren's syndrome with sialography and scintigraphy, and indicated that ultrasonography showed the best performance followed by sialography and scintigraphy<sup>11</sup>. Considering convenience, non-invasiveness, and inexpensiveness, employing the ultrasonographic examination has advantages over the salivary secretion test and scintigraphy. The study here showed comparatively high false positive results with the ultrasonographic examination. One reason may be that the organs examined by ultrasonography include the bilateral parotid and submandibular glands and that the diagnosis of SS was made based on the findings of these four glands. If one of the glands presents a finding which meets SS criterion, the case was diagnosed as SS. Meanwhile, the histopathological examination showed high false negative results, it maybe because the diagnostic criterion of the biopsy is quantitative, and cases such as Gr2 where the diagnosis may be in error were considered as negative SS. To avoid bias and technical errors related to the

histological procedure, a multilevel examination of the biopsy sample is recommended<sup>15</sup>. The interposition of 200  $\mu$ m between the evaluated sections is sufficient and a minimum of three different section levels are required to score the focus independently. Comparing the multilevel examination of the minor salivary gland biopsy with the American-European Consensus Group criteria, sensitivity was not affected, while specificity and accuracy increased 9.8% and 5.9%, respectively. This improvement was mostly due to increased specificity in biopsies with a baseline  $1 < FS < 2$  and  $FS > 2$ <sup>15</sup>. This study showed that the correlation between sialography and ultrasonography was higher than the correlations with the other tests. The differences in correlation may arise as the sialographic and ultrasonographic examinations investigate the same glands (the parotid and/or submandibular glands), while the histopathological examination investigates minor salivary glands of the lip. For the reason above, the parotid gland biopsy should be considered, although it is not the organ that yields the criteria for SS. Pijpe, et al. compared the parotid gland biopsy with labial biopsy, and found comparable sensitivities and specificities<sup>13</sup>. The parotid gland biopsy does not result in loss of motor function or permanent sensory loss, while labial biopsy led to permanent sensory loss in 6% of the patients. Pijpe, et al<sup>16</sup>. concluded that the diagnostic potential of the parotid biopsy is comparable with that of the labial biopsy in the diagnosis of SS, additionally it offers the potential of detecting malignant lymphomas. Approximately 50% of the non-SS patients in the study here reported dry mouth symptoms. In addition to SS, dry mouth can be induced by the effects of aging, medication, systemic conditions such as diabetes mellitus and psychological effects<sup>17,18</sup>. It is also reported that lifestyle patterns (alcohol) and mouth breathing are the causes of dry mouth<sup>19,29</sup>. Sialography is the conventional examination for salivary gland complaints, furthermore, it is the most reliable modality, especially for SS. However, sialography is invasive because of the necessity of cannulation and injection of contrast materials and it requires radiation exposure. The authors have experienced two cases of complications associated with the sialography, temporary sialadenitis resulting from cannulation and injection of the contrast materials, which were resolved with antibiotics therapy. Cannulation of the main duct is sometimes difficult especially when the orifices cannot be identified due to atrophy and/or hyposalivation. Here the diagnostic accuracy depends on the observer, and inter-observer agreement of the diagnosis of sialiectasia varied from poor to good with trained and expert observers. The technique and diagnosis of sialography lacks general applicability and requires specific expertise<sup>3</sup>. This study, similar to the other study, indicated that the diagnostic reliability of sialography for SS is better

than other diagnostic tools<sup>3</sup>. Furthermore, its costs are low and it has a relatively low degree of invasiveness, as well as it is a relatively simple and quick procedure. Sialography is an especially useful tool in monitoring the progress of SS due to the similarities in the progress of sialectasia. MR is a noninvasive examination of the salivary glands, and Izumi et al.<sup>21</sup> reported that a quantitative analysis of MR images for the standard deviation (SD) of the signal intensity is useful in the diagnosis of SS<sup>18</sup>. They indicated that the signal intensity on T1-weighted MR images of parotid gland in patients with SS increased corresponding to the progression of the disease which represents fat deposits. The MR examination is more expensive to conduct than sialography and contraindicated in patients with claustrophobia, cardiac pacemakers, and with metal tooth crown restorations and prosthesis. Ultrasonography is not included among the diagnostic criteria for SS, but the diagnostic reliability is similar to histopathology and sialometry combined with scintigraphy, which are components of the examination. Shimizu et al.<sup>22</sup> reported that characteristic sonographic findings (multiple hypoechoic areas, multiple hyperechoic lines and/or spots, multiple hypoechoic areas surrounded with hyperechoic lines and/or spots) could differentiate positive cases of the Sjögren' s syndrome from negative controls to a very significant degree, and that the findings correlated well with the sialographic grade. Niemela et al.<sup>23</sup> evaluated ultrasonography of salivary glands in primary Sjogren' s syndrome and compared ultrasonography with parotid magnetic resonance (MR) imaging and MR sialography. They reported that MR sialography was the most sensitive method (96%), followed by MR imaging (81%) and US (78%), and the specificity of US was 94%. Recently, saliva has attracted interest as a biomarker and in sialochemistry as a non-invasive means of diagnosing SS. Kalk, et al.<sup>24</sup> reported that the parotid sodium and chloride concentrations combined with the stimulated submandibular and sublingual gland saliva flow rate was the most accurate test for SS, showing a sensitivity of 85% and a specificity of 96%. Hammi, et al.<sup>25</sup> compared the sensitivity of parotid saliva to that of serum in detecting anti-SSA/Ro and anti-SSB/La auto-antibodies in patients with SS, and indicated that serum was significantly more sensitive than saliva in detecting SSA/Ro and SSB/La antibodies (P=0.001). Ultrasonography is similarly non-invasive, furthermore, inexpensive, concise and a real-time examination. In conclusion, considering the higher correlation to sialography, ultrasonographic examination can be an alternative modality to histopathology, and included as a global diagnostic tool for SS.



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## LEGENDS

### Figure. 1.

Sialograms of the parotid glands. **A**, Sialogram of normal parotid gland (stage 0). **B**, Sialogram of stage 1. Punctate sialectasia, less than 1 mm in size is observed.

### Figure. 2

Sonograms of the parotid glands scanned parallel to the occlusal plane (left: posterior, right: anterior). **A**, Sonogram of normal parotid gland (Grade 0). Internal echoes are homogeneous. **B**, Sonogram of parotid gland of SS (Grade 3). Internal echoes are evident inhomogeneous. Multiple scattered hypoechoic areas (2–6 mm) are observed.

### Figure. 3

Histology of the labial glands of a SS patient. More than one focus per 4 mm<sup>2</sup> are observed (grade 4).

### Figure. 4

Receiver operating characteristic (ROC) curves in the differential diagnoses of non-Sjögren syndrome (SS) and SS groups. Sialography showed a superior accuracy to both ultrasonography and pathology.

TPF, true positive fraction; FPF, false positive fraction.

**Table.1 Revised Japanese criteria for Sjogren' s syndrome (1999)**

1. **Histopathology** : Positive for at least 1 of (A) or (B)
  - (A) Focus score, 1 (50 or above periductal lymphoid cell infiltration) or above in a 4mm<sup>2</sup> minor salivary gland biopsy
  - (B) Focus score, 1 (50 or above periductal lymphoid cell infiltration) or above in a 4mm<sup>2</sup> minor lacrimal gland biopsy
2. **Oral examination** : Positive for at least 1 of (A) or (B)
  - (A) Abnormal findings in sialography, Stage 1 or above
  - (B) Decreased salivary secretion (flow rate, 10ml or below per 10 min according to chewing gum test or 2g or below per 2min according to the Saxon test) and decreased salivary function according to salivary scintigraphy
3. **Ocular examination** : Positive for at least 1 of (A) or (B)

Schirmer' s test, 5mm or below per 5 min; and in the rose bengal test, 3 or above according to van Bijsterveld score

  - (B) Schirmer' s test, 5mm or below per 5 min and positive fluorescein staining test
4. **Serological examination** : Positive for at least 1 of (A) or (B)
  - (A) Anti-Ro / SS-A antibody
  - (B) Anti-Ro / SS-B antibody

**Diagnostic criteria : Diagnosis of Sjogren' s syndrome can be made when the patient meets at least 2 of the above 4 criteria.**

Table.2 The sialographic classification of Rubin and Holt (1957)

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Classification	Sialographic findings
Stage 0 (normal)	no contrast media collection
Stage 1 (punctate)	contrast media collection 1 mm in diameter or smaller
Stage 2 (globular)	contrast media collection 1-2 mm in diameter
Stage 3 (cavitary)	contrast media collection 2 mm in diameter or larger
Stage 4 (destructive)	complete destruction of the gland parenchyma

**Table.3 Grading of ultrasonography of Salaffi, et al. (2000)**

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<b>Grade</b>	<b>Findings</b>
0 (homogeneity)	Normal glands
1 (slight inhomogeneity)	Small hypoechoic spots
2 (mild inhomogeneity)	Multiple scattered hypoechoic areas (< 2mm)
3 (evident inhomogeneity)	Multiple hypoechoic areas (2-6 mm)
4 (gross inhomogeneity)	Multiple hypoechoic areas (>6 mm)

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Table.4 Grading of labial salivary gland biopsies of Greenspan et al. (1974)

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Grade	Lymphocytes and plasma cells per 4 mm <sup>2</sup>
0	Absent
1	Slight infiltrate
2	Moderate infiltrate or less than one focus per 4 mm <sup>2</sup>
3	One focus per 4mm <sup>2</sup>
4	More than one focus per 4 mm <sup>2</sup>

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Focus, according to Waterhouse and Doniach, is an aggregate of fifty or more lymphocytes, histiocytes, and plasma cells.

**Table.5 Comparison of diagnostic reliability of sialography and US and pathology**

<b>%</b>	<b>Sensitivity</b>	<b>Specificity</b>	<b>Accuracy</b>
<b>Sialography</b>	<b>83.3*</b>	<b>94.6*</b>	<b>89.0*</b>
<b>US</b>	<b>77.8</b>	<b>78.4*</b>	<b>78.1*</b>
<b>Pathology</b>	<b>63.9*</b>	<b>91.9</b>	<b>78.1*</b>

**\* : P < 0.05 (chi-square test)**



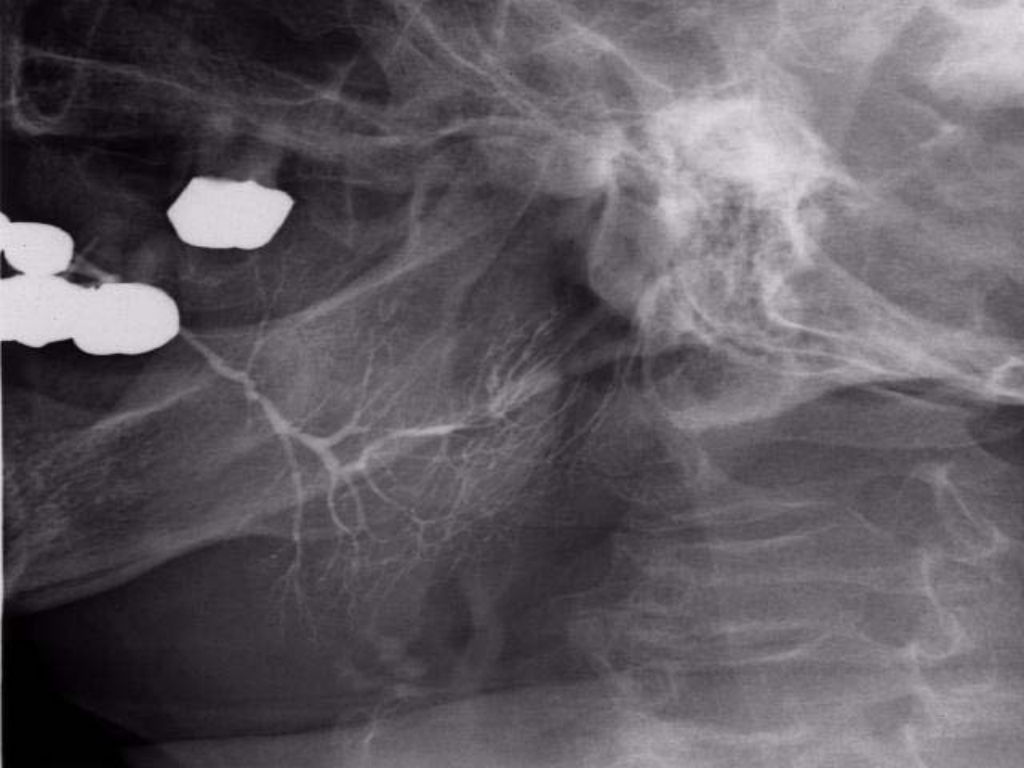
**Table.6 Correlation between diagnostic tools**

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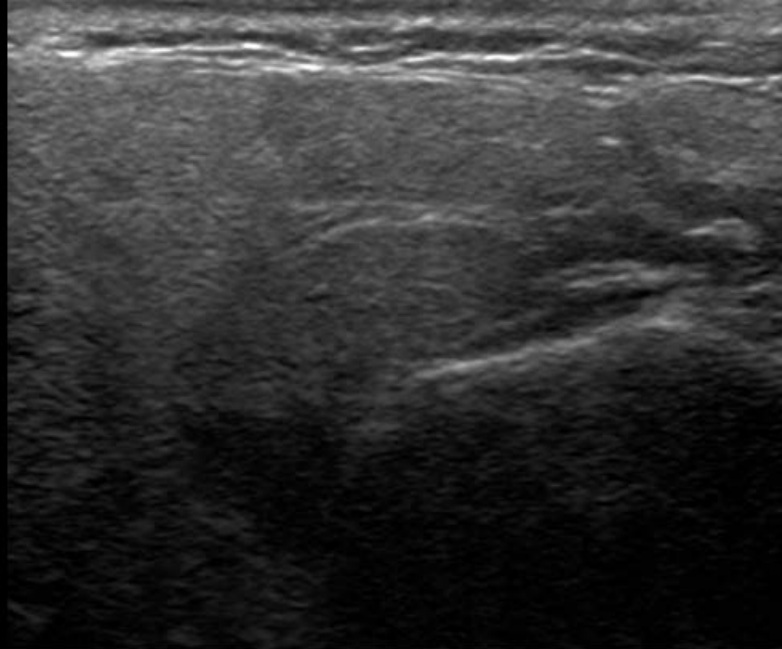
Sialo - US       $r = 0.58$   
Sialo - Patho    $r = 0.35$   
US - Patho       $r = 0.50$

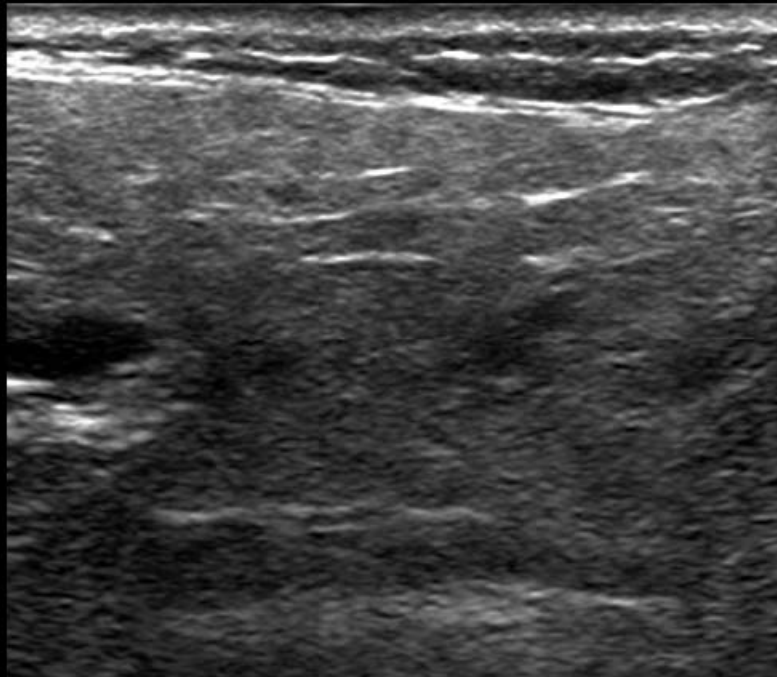
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<b>r</b>	
<b>0.0~0.25</b>	<b>little correlation</b>
<b>0.25~0.5</b>	<b>slight correlation</b>
<b>0.5~0.75</b>	<b>good correlation</b>
<b>0.75~1.0</b>	<b>strong correlation</b>









G

