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Cut-off value for 25 Hydroxy-vitamin D which leading to symptomatic vitamin D deficiency in children is 15 ng/mL (37.5 nmol/L) in a CLIA.

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Introduction

Vitamin D is one of the essential nutrients which is important for bone and calcium metabolism. Its deficiency can lead to rickets and hypocalcemia during childhood. Global consensus¹⁾ stated the level of 25 hydroxy-vitamin D (25OHD) as insufficient and deficient as follows;

Vitamin D insufficiency : 12-20 ng/mL (30-50 nmol/L)
Vitamin D deficiency : < 12 ng/mL (30 nmol/L)

However, some children don't become symptomatic even though their 25OHD level were low. Indeed, the evidence levels of the above ranges are not so high. For example, vitamin D deficiency, based on parathyroid hormone (PTH) elevation, was defined as a 25OHD level of less than 13.6 ng/mL (34 nmol/L) (Figure 1)²⁾.

This study aimed to determine the cut-off value of 25OHD for symptomatic vitamin D deficiency by doing receiver operating characteristic (ROC) curve analysis.

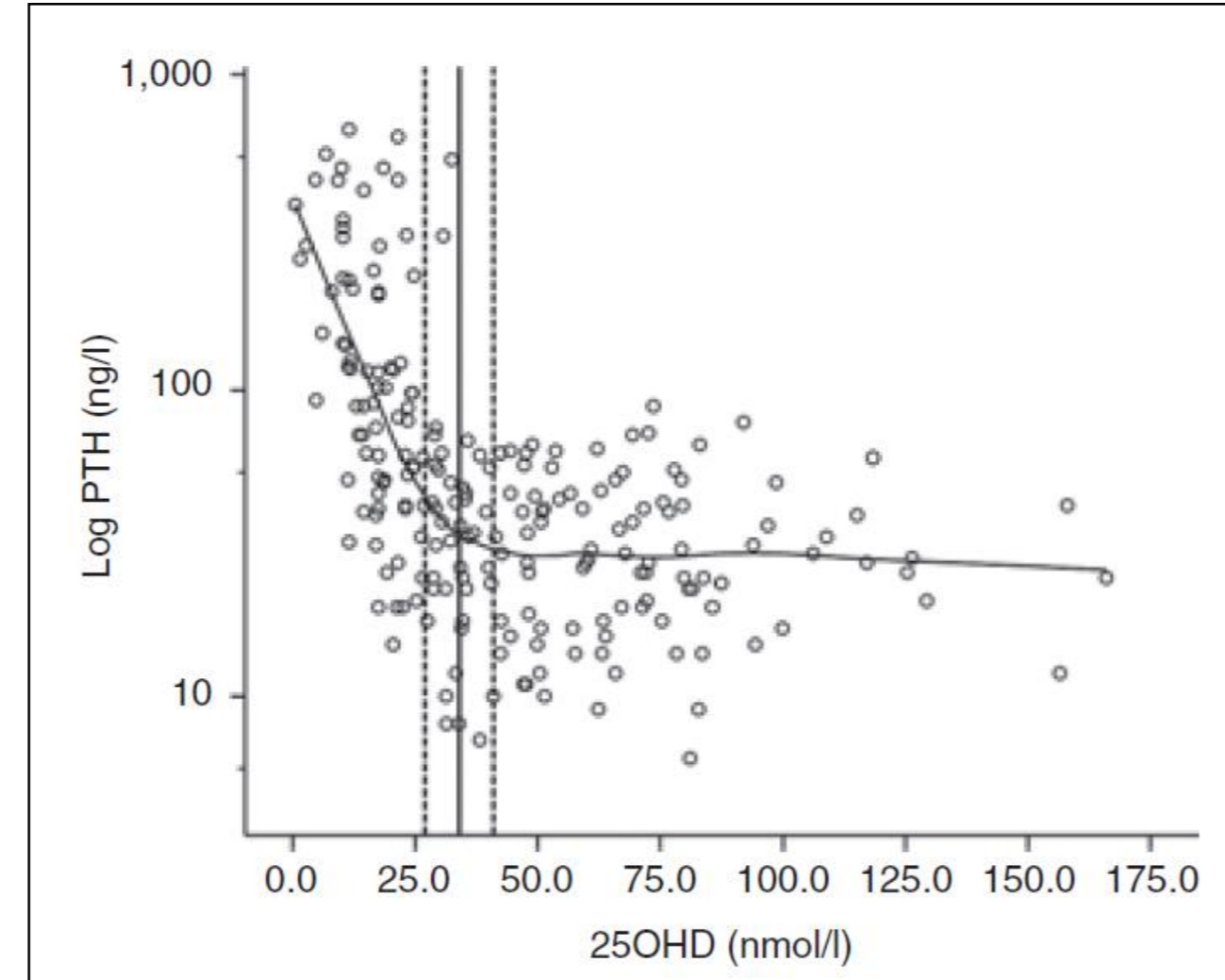


Figure 1. Inverse relationship between log PTH and 25OHD²⁾

Subjects and Methods

Design

Retrospective study using the electric medical records

Setting

April 2013-March 2018 at four hospitals
Tokyo Metropolitan Children's Medical Center, Niigata University Medical and Dental Hospital,
Sapporo Medical University Hospital, Osaka City General Hospital

Participants

Children aged 0 to 15 years old who received screening for vitamin D deficiency including measurements of 25OHD

Main Outcome

Cut-off value of 25OHD leading to symptomatic vitamin D deficiency

Exclusion criteria

- Receiving supplementation such as vitamin D, Calcium or Phosphorus formulation
- Syndromes with hypoparathyroidism such as HDR syndrome, 22q11.2 deletion syndrome
- Hypophosphatemic rickets
- Drug-induced hyperphosphatasemia/rickets
- Renal insufficiency (including hemodialysis)
- Transient hyperphosphatasemia
- Hypophosphatasia
- Bow legs due to orthopedic disorder

Diagnosis of rickets and hypocalcemia due to vitamin D deficiency³⁾

Rickets : Diagnosed by radiographic signs of wrist or knee + high levels of alkaline phosphatase (ALP) (metaphyseal fraying, cupping, epiphyseal widening)

Healing rickets : Diagnosed by Radiographic signs (the zone of provisional calcification) without biochemical abnormality

Hypocalcemia : Serum corrected calcium by albumin was lower than 8.4 mg/dL (2.1 mmol/L)

Other causes of rickets or hypocalcemia due to vitamin D deficiency were excluded

Measures

The following data were further collected for each subject:

Serum levels of 25OHD, calcium (Ca), phosphate (P), ALP, albumin, intact parathyroid hormone (PTH), urine levels of calcium and creatinine (Cr), radiographs of wrists or knee.

There were 4 methods of 25OHD measurements; Radio Immunoassay (RIA), Double-antibody Radioimmunoassay (RIA2), Chemiluminescent Immunoassay (CLIA), Chemiluminescent Enzyme Immunoassay (CLEIA). Therefore we converted the values measured by methods other than CLIA to values by CLIA.

Statistical analysis

All statistical analyses were performed with EZR⁴⁾ (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). All descriptive results are expressed as median and minimum to maximum values. Because of the skewed distribution of the outcome measurements, pair-wise Mann-Whitney U test were used to compare age, 25OHD, U-Ca/Cr and PTH. A p value of less than 0.05 was considered statistically significant. We measured sensitivity and specificity values under a receiver operating characteristic (ROC) curve to find out whether 25OHD measurements were effective in the presence of rickets or hypocalcemia.

Discussion & Conclusion

- The 25OHD cut-off value for leading to symptomatic vitamin D deficiency was 15.0 ng/mL in a CLIA.
- U-Ca/Cr ratio of symptomatic cases was lower than asymptomatic cases when 25OHD level was less than 15.0 ng/mL

To the best of our knowledge, this is the first study to investigate the 25OHD cut-off value for symptomatic vitamin D deficiency. However, we could not evaluate all data due to retrospective study. Furthermore, our results can't be applied to other countries because we conducted in a single ethnic group.

Miyai et al. reported U-Ca/Cr ratio could be influenced by the supply of calcium and that ratio less than 0.1 (mg/mg) was useful for detecting secondary hyperparathyroidism in patients with vitamin D deficiency (Figure 4)⁵⁾. Our result suggests that nutritional education for calcium intake or calcium supplementation may be one of treatment of vitamin D deficiency in addition to active vitamin D (i.e., alfacalcidol) in cases with low U-Ca/Cr ratio. A prospective trial of Ca intervention in this aspect could be planned.

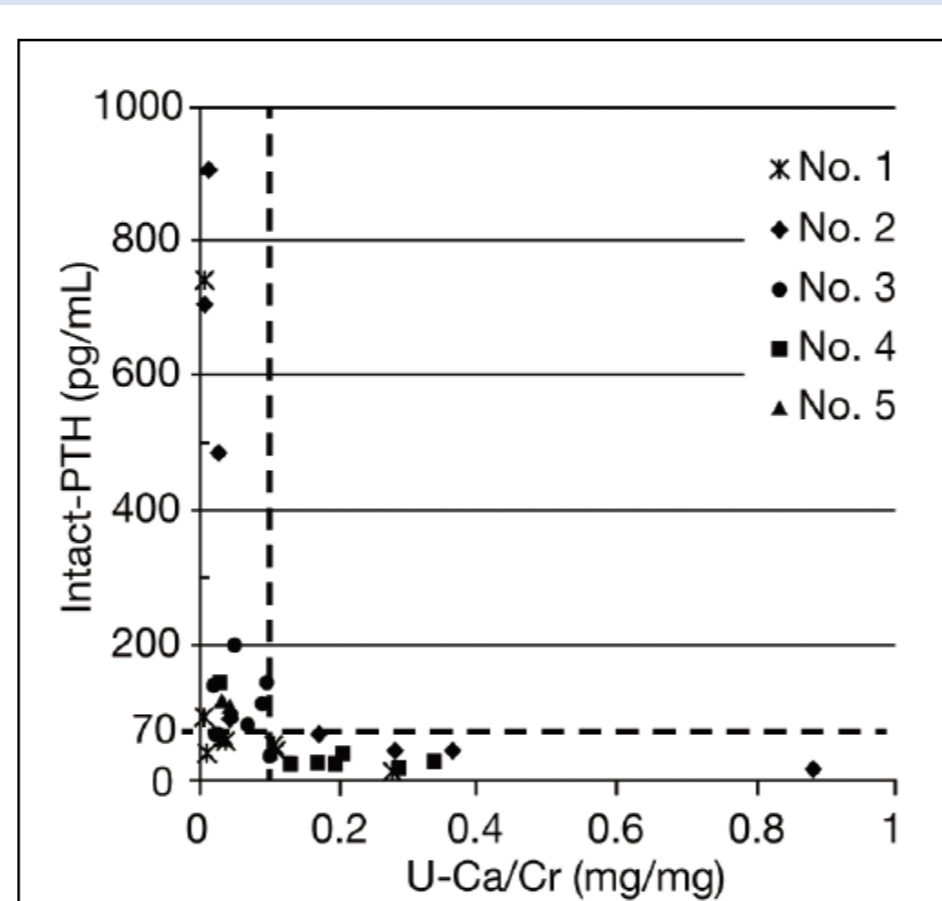


Figure 4. Relationship between Intact-PTH and U-Ca/Cr in vitamin D deficiency patients

Results

Characteristics

All cases (n=197) 25OHD:13.4 ng/mL (3.1-36.1)			
Asymptomatic (n=117)	Healing Rickets (n=11)	Symptomatic (n=69)	
18.7 ng/mL (4.0-36.1)	12.1 ng/mL (6.6-31.7)	7.4 ng/mL (3.1-15.5)	
		Rickets (n=53)	8.4 ng/mL (4.1-15.5)
		Rickets and Hypocalcemia (n=9)	6.0 ng/mL (3.1-10.8)
		Hypocalcemia (n=7)	4.4 ng/mL (3.1-8.6)

Table 1. Diagnosis and median 25OHD levels of each cases.

There were 605 patients who were tested 25OHD. 408 were excluded and 197 patients were surveyed in this study (Table 1). 117 were asymptomatic, 11 were healing rickets, and 69 were symptomatic. Healing rickets were excluded for the further analysis below since the definition is still controversial.

25OHD

25OHD levels are shown on the Box and Whisker Plot for asymptomatic and symptomatic cases and there was significant difference between them (Figure 2).

In ROC curve analysis, the cut-off value for 25OHD between the asymptomatic (n=117) and symptomatic (n=69) cases was 15.0 ng/mL with a sensitivity and specificity of 73% and 99%, respectively. (Figure 3)

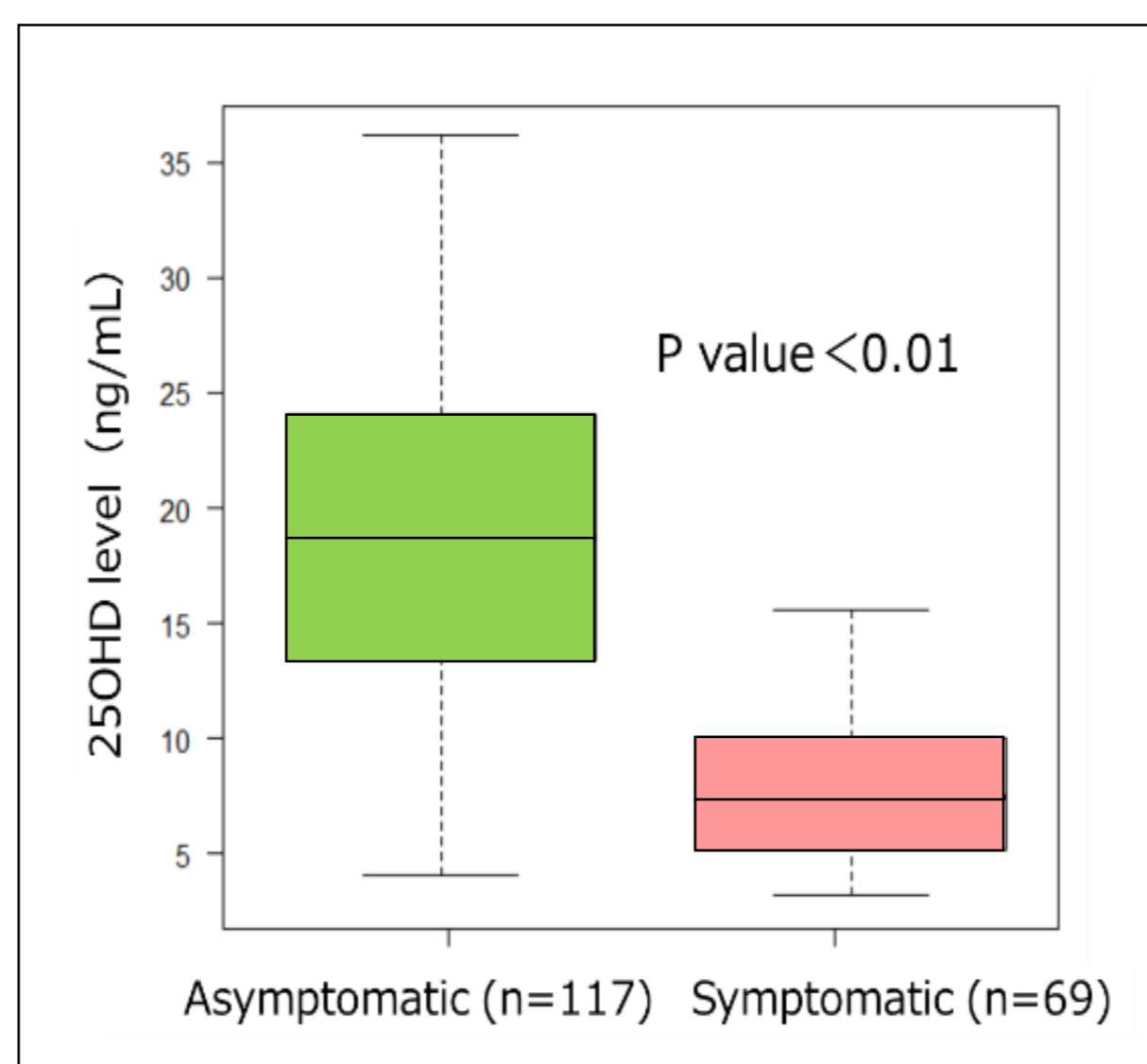


Figure 2. Box and whisker plot graphs for asymptomatic and symptomatic.

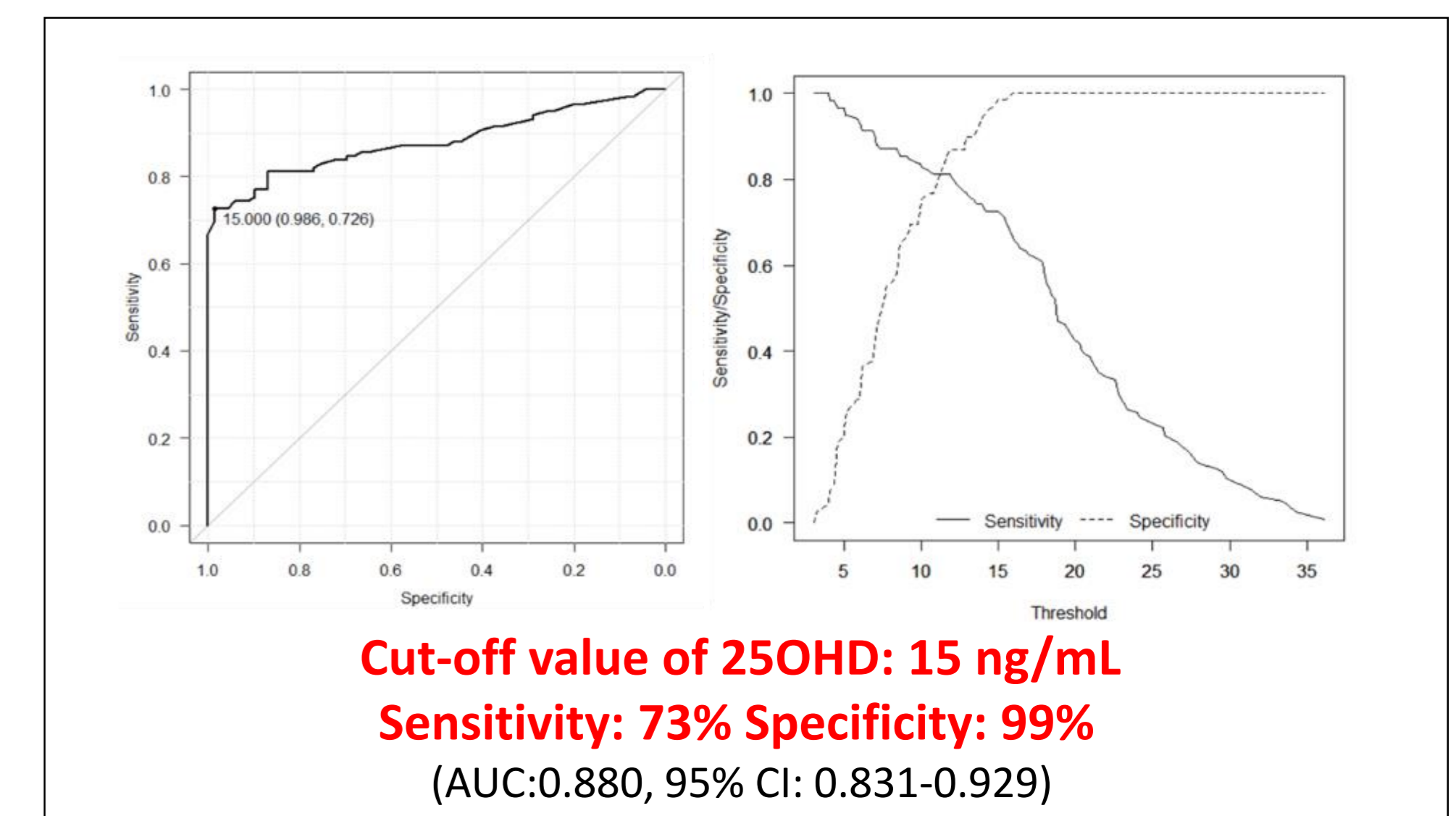


Figure 3. The ROC curve for 25OHD level in the presence of vitamin D deficiency

The factor associated with symptomatic vitamin D deficiency

In our study, there were 102 cases whose 25OHD levels were less than 15. However, 34 of 102 cases were asymptomatic. To find a predictor of symptomatic vitamin D deficiency, we compared clinical data of asymptomatic cases with those of symptomatic (Table 2). When 25OHD level were less than 15, there was no statistical difference in the level of 25OHD between asymptomatic and symptomatic cases.

It is well known that ALP, and PTH elevate in vitamin D deficiency due to secondary hyperparathyroidism. Therefore, we especially focused on the U-Ca/Cr ratio, which was 0.16 and 0.03 in asymptomatic and symptomatic cases, respectively. Those were significantly different. All the analyses were similarly done after excluding cases with hypocalcemia, giving the same statistical significance. (Table 3)

	25OHD ≤ 15 ng/mL (n=102)		P value
	Asymptomatic (n=34)	Symptomatic (n=68)	
Age: month (n=102)	9 (1-175)	18 (1-143)	0.146
25OHD : ng/mL (n=102)	8.8 (4.0-15.0)	7.4 (3.1-14.7)	0.11
Ca : mg/dL (n=102)	9.9 (8.7-10.9)	9.3 (5.6-10.9)	< 0.05
P : mg/dL (n=102)	5.0 (2.8-7.5)	4.3 (3.5-6.2)	< 0.05
ALP : IU/L (n=102)	1430 (301-4492)	2232 (666-9044)	< 0.05
Intact-PTH: pg/mL (n=96)	50.5 (9-483)	222.5 (36-776)	< 0.05
U-Ca/Cr ratio (n=78)	0.16 (undetectable-1.32)	0.03 (undetectable-0.43)	< 0.05
U-Ca/Cr ratio (n=63) ※excluding ≤ 6 month	0.135 (undetectable-0.47)	0.03 (undetectable-0.41)	< 0.05

Table 2. Comparison of clinical data between asymptomatic and symptomatic cases whose 25OHD levels were less than 15.0 ng/mL.

	25OHD ≤ 15 ng/mL (n=86) ※excluding cases with hypocalcemia		P value
	Asymptomatic (n=34)	Symptomatic (n=68)	
25OHD : ng/mL (n=86)	8.8 (4.0-15.0)	7.8 (4.1-14.7)	0.49
U-Ca/Cr ratio (n=60)	0.16 (undetectable-1.32)	0.01 (undetectable-0.41)	< 0.05
U-Ca/Cr ratio (n=53) ※excluding ≤ 6 month	0.135 (undetectable-0.47)	0.03 (undetectable-0.41)	< 0.05

Table 3. Comparison of clinical data between asymptomatic and symptomatic cases after exclusion of cases with hypocalcemia whose 25OHD levels were less than 15.0 ng/mL.

References

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