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Response of preterm infants with transient hypothyroxinemia of prematurity to the thyrotropin-releasing hormone stimulation test is characterized by a delayed decrease in thyroid stimulating hormone after the peak

TRH tests on VLBW infants with THOP

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Abstract

Objectives

We evaluated the response to the thyrotropin-releasing hormone (TRH) stimulation test in very low birth weight (VLBW) infants to elucidate the etiology of transient hypothyroxinemia of prematurity (THOP).

Design and Methods

We performed TRH stimulation tests on 43 VLBW infants. Subjects were divided into two groups; a THOP group (N=11; basal TSH < 15 mU/L and basal FT4 $\leq$ 0.8 ng/dL) and a Non-THOP group (N=32; basal TSH < 15 mU/L and basal FT4 > 0.8 ng/dL). Basal FT4 and FT3 were measured before, and TSH (0, 30, 60, 90, 120, and 180 min) was measured after, the administration of TRH (7 μ g/kg). We calculated the ratio of TSH 180 min to TSH 0 min as the primary outcome. We also collected data on T3 and rT3 in this study.

Results

In both groups, TSH 30 min values were the highest. However, the ratios of TSH 180 min to TSH 0 min in the Non-THOP group and the THOP group were (median [IQR]) 1.3 [1.0-1.7] and 3.0 [1.5-5.3] ($p<0.01$). No significant differences were observed in T3 (1.0 [0.8-1.3] and 0.7 [0.4-0.7] ng/mL, $p=0.06$). However, in the THOP group, rT3 was significantly lower than that of the Non-THOP group (168.0 [148.1-197.0] and 92.9 [74.7-101.6] pg/mL, $p<0.01$).

Conclusions

The delayed decrease in the TSH concentration after the peak for the TRH tests and decreased levels of rT3 suggest that the main etiology for THOP is suppression at the level of the

hypothalamus, but not inactivation of peripheral thyroid hormone metabolism.

Key words

transient hypothyroxinemia of prematurity

thyrotropin-releasing hormone stimulation test

very low birth weight infants

hypothalamic-pituitary-thyroid axis

non-thyroidal illness syndrome

thyroid-stimulating hormone

reverse triiodothyronine

Background

Transient hypothyroxinemia of prematurity (THOP) is a condition that is frequently seen in very low-birth weight (VLBW) infants, especially in extremely preterm (< 28 weeks gestation) and very preterm infants (28-32 weeks).¹⁻⁴ THOP is defined by temporary low levels of thyroxine (FT4 or T4) and triiodothyronine (FT3 or T3) serum concentrations, in combination with normal or low levels of thyroid-stimulating hormone (TSH).²⁻⁴ The etiology of THOP is considered to be multifactorial, including iodine deficiency, the withdrawal of maternal-placental T4 transfer, immaturity of the hypothalamic-pituitary-thyroid (HPT) axis, impaired synthesis and peripheral metabolism of iodothyronines, non-thyroidal illness, and drugs that interfere with the HPT axis.^{2,5}

Thyroid hormone is essential for development of the central nervous system and psychomotor development.⁶ To sustain postnatal development of VLBW infants, maintaining serum T4 and T3 levels in cases suspected of permanent primary hypothyroidism and true central hypothyroidism are important.

Measurement of TSH in infants with hypothyroxinemia is useful to detect primary hypothyroidism.⁷⁻¹⁰ In VLBW infants, transient hypothyroidism may be accompanied by a delayed rise in the TSH level that is not detected until several weeks after birth.^{7,11} Several investigators have recommended screening of thyroid function of VLBW infants at two to six weeks of age.^{8,11,12}

On the other hand, in cases with low levels of (F)T4 and normal or low levels of TSH, it is difficult to distinguish those with THOP from those with true central (secondary, hypopituitary, or hypothalamic) hypothyroidism.^{12,13} The assessment of thyroid function in VLBW infants with THOP is difficult. Systemic illness decreases serum thyroxine binding globulin (TBG) and T4 then inhibits extrathyroidal conversion of T4 to T3.¹² In addition, drugs, such as catecholamine

and glucocorticoids, decrease pituitary secretion of thyrotropin.³ Thus, sick VLBW infants could have low levels of T4 and T3.

Furthermore, whether THOP should be treated is still controversial.^{12,14-16} Some physicians consider LT4 supplementation therapy is necessary due to an immature HPT axis in infants that results in an inability to produce adequate TSH levels¹⁷⁻¹⁹, but others reported no positive effects in relation to thyroid hormone treatment^{6,20,21}.

In some cohort studies, the results of a comparison of longitudinal changes in TSH and FT4 in VLBW infants with THOP after LT4 supplementation therapy were reported; infants in the treated group showed significantly lower mean plasma TSH levels after treatment with no significant change in mean FT4 levels^{6,21,22}. These results indicate LT4 supplementation is regulated by the HPT axis in infants with THOP. Not only immaturity, but also some other self-regulated systems, may be involved in this phenomenon.

Moreover, in recent reports, circulatory dysfunction associated with LT4 administration to VLBW infants has been reported^{23,24}. These reports suggested that LT4 supplementation to infants with THOP requires more careful attention. Clarifying the etiology of THOP may lead to improvements over the present strategy in the management of THOP.

Some researchers have recommended TRH stimulation tests in cases with low (F)T4 levels without elevation of TSH levels to distinguish true central hypothyroidism from mild primary hypothyroidism^{13,25-27}. The current mass-screening system using only TSH and T4 is not accurate enough to evaluate borderline status, including THOP²⁸. Therefore, we performed TRH stimulation tests in all VLBW infants admitted to our NICU, in order to assess the status of their HPT axis function. In our previous studies, we performed the TRH stimulation test at ~ two weeks of age and measured TSH at 0 min and 30 min after the administration of 7 µg/kg TRH in VLBW infants.

We reported that basal increased TSH levels (TSH > 10 mU/L) and a hyperresponse to TRH tests (peak TSH > 45 mU/L) suggested subclinical thyroid dysfunction and serum TSH values at about two weeks of age could be useful for the prediction of delayed TSH elevation²⁹. Based on these results, we administer levothyroxine (LT4) to infants with basal TSH > 15 mU/L or infants with basal TSH 10-15 mU/L and a hyperresponse to TRH tests (peak TSH > 45 mU/L).

We also performed TRH stimulation tests on preterm infants (30 gestational weeks or less) at two weeks of age and showed that TSH values at 30 min were not significantly different between the THOP group and control group³⁰. The results suggested the TSH response to TRH stimulation tests in VLBW infants with THOP was not comparable with that of euthyroid infants. In addition, pituitary gland dysfunction may not be the main cause of THOP. However, we could not evaluate the hypothalamus function only from the TSH value at 30 min after the TRH stimulation tests. In order to clarify the pathophysiology of THOP, including an evaluation of hypothalamus function, we performed 180 min TRH stimulation tests in this study. TSH levels (0, 30, 60, 90, 120, and 180 min) after administration of 7 µg/kg of TRH were measured. In addition, we measured rT3 and T3 in order to assess the peripheral metabolism of infants with THOP.

Methods

Subjects

The subjects were 47 VLBW infants (< 37 weeks gestational age) admitted to the NICU of Kyoto University Hospital between November 2014 and June 2016. We excluded infants with maternal thyroid diseases, chromosomal abnormalities and other suspicious hypopituitarism (including ambiguous genitalia, hypoglycemia due to pituitary deficits and mid-line anomalies). One severe hypoxic-ischemic encephalopathy (HIE) case was excluded because of suspicious hypopituitarism. Three infants with elevated TSH (>15 mU/L) who were administered levothyroxine (LT4) were excluded on the grounds that they were diagnosed as subclinical thyroid dysfunction. Finally, a total of 43 VLBW infants were recruited and underwent TRH stimulation tests at about two weeks of age (Figure 1).

Ethics approval was obtained from the Kyoto University Graduate School and Faculty of Medicine Ethics Committee, and parental informed consent was obtained for all subjects.

TRH stimulating tests

Serum FT4, FT3, and TSH were measured by ECLIA (Roche Diagnostics, Tokyo, Japan) at a postnatal age of approximately two weeks. We measured TSH levels during the TRH stimulating test by the filter paper spot TSH method with ENZAPLATE Neo-TSH™, an enzyme-linked immunosorbent assay kit (Siemens Healthineers Japan, Tokyo, Japan) and obtained the TSH values (0, 30, 60, 90, 120, and 180 min) after administration of 7 µg/kg of TRH (Tanabe Mitsubishi Pharmaceutical Company, Osaka, Japan). There was a good correlation between the basal TSH levels measured with ECLIA and the TSH 0 min levels measured with the filter paper spot TSH method ($r = 0.79$, $p < 0.0001$, Figure 2). We also measured serum T3 by ELISA (Abnova Diagnostics, Tokyo, Japan) and reverse triiodothyronine (rT3) by ELISA (CUSABIO

TECHNOLOGY LLC, China).

Statistical analysis

Based on the distribution, continuous variables were described by the median (interquartile range) and compared by Wilcoxon's rank sum test between the Non-THOP and THOP groups. Categorical variables were expressed with a number (%) and compared by Fisher's exact test. Spearman's rank correlation analysis was used to evaluate the correlation between the basal TSH levels measured with ECLIA and the TSH 0 min levels measured with the filter paper spot TSH method. All reported p -values were two-sided and p -values less than 0.05 were considered significant. All analyses were calculated with JMP Pro 14 (SAS Institute Japan).

Results

Subjects profiles

In this study, a total of 43 VLBW infants (< 37 weeks gestational age) were recruited and underwent TRH stimulation tests at two weeks of age (Figure 1). They were divided into two groups: a THOP group (N=11; basal TSH < 15 mU/L and basal FT4 $\leq$ 0.8 ng/dL (10.3 pmol/L)) and a Non-THOP group (N=32; basal TSH < 15 mU/L and basal FT4 > 0.8 ng/dL). We set the FT4 cut-off value at 0.8 ng/dL referring to past articles³¹⁻³³.

The characteristics of the two groups are shown in Table 1. In the THOP group, the gestational age (GA), birth weight (BW), and birth length were significantly different from those in the Non-THOP group. Also, infants in the THOP group were much more likely to be ventilated at the time of the TRH stimulation tests than infants in the Non-THOP group (40.6% (13 events/32 subjects), and 100% (11/11), $p<0.01$) and tended to receive less enteral feeding at the time of the TRH stimulation tests (107.0 [77.3-137.0] and 29.0 [15.0-91.0] ml/kg/day, $p<0.01$). On the other hand, as for the rate of postnatal glucocorticoids and catecholamine use from at birth to the time of the TRH stimulation test, there were no significant differences between the Non-THOP group and the THOP group ($p=0.28$ and $p=0.67$, respectively).

Basal FT4 and FT3 values at two weeks of age

Basal FT4 values in the Non-THOP group and the THOP group were 1.5 [1.1-1.7] and 0.7 [0.6-0.8] ng/dL ($p<0.01$). Basal FT3 values were 2.1 [1.9-2.4] and 1.0 [0.8-1.1] ng/dL ($p<0.01$).

TRH stimulation test

The corrected age at the time of the TRH stimulation tests was younger in the THOP group than that in the non-THOP group, (30.3 [29.2-32.0] weeks and 28.0 [27.1-29.0] weeks, $p<0.01$). In both

groups, TSH 30 min values were the highest (21.7 [15.8-33.3] and 23.5 [22.6-40.2] mU/L, $p=0.37$). However, in the THOP group, the response to the TRH stimulation test was characterized by a delayed decrease in TSH after the peak (Table 2). TSH 90 min, TSH 120 min, and TSH 180 min values for infants in the THOP group were significantly higher than those of the Non-THOP group (14.4 [11.4-22.1] and 22.2 [15.8-30.2] mU/L ($p<0.05$), 11.3 [9.5-15.9] and 18.7 [12.1-18.7] mU/L ($p<0.05$), and 8.6 [6.5-11.0] and 14.1 [9.2-16.4] mU/L ($p<0.05$), respectively). The ratio of TSH 180 min to TSH 0 min in the THOP group was significantly higher than that of the Non-THOP group (1.3 [1.0-1.7] and 3.0 [1.5-5.3], $p<0.01$) (Table 2).

Thyroid hormone profiles (N=26) and a comparison between those of the Non-THOP and THOP groups

We also collected data on T3, rT3, the rT3/T3 ratio, and the rT3/FT4 ratio in 26 VLBW infants (Table 3). In the THOP group (N=6), the GA and birth BW were significantly different from those in the Non-THOP group (N=20). In the THOP group, all of the infants were ventilated at the time of the TRH stimulation tests (9/20 (45.0%) and 6/6 (100%), $p<0.05$). As for the thyroid hormone profiles, both basal FT4 and basal FT3 levels were significantly lower in the THOP group (1.3 [1.1-1.6] and 0.6 [0.6-0.8] ng/mL ($p<0.01$), 2.1 [1.9-2.3] and 1.0 [0.7-1.2] ng/mL ($p<0.01$), respectively). The difference in T3 levels between the Non-THOP group and the THOP group was not significant (1.0 [0.8-1.3] and 0.7 [0.4-0.7] ng/mL, respectively, $p=0.06$).

rT3 levels of the Non-THOP and THOP groups were 168.0 [148.1-197.0] and 92.9 [74.7-101.6] pg/mL, respectively. rT3 levels were significantly lower in the THOP group than in the Non-THOP group ($p<0.01$). However, no significant differences were observed in the rT3/T3 ratio or the rT3/FT4 ratio between the Non-THOP group and the THOP group (180.2 [136.0-223.7] and 139.3 [74.2-222.6] ($p=0.21$) and 130.5 [113.2-152.4] and 120.1 [108.5-173.2] ($p=0.98$), respectively).

DISCUSSION

Recent progress in perinatal and neonatal medicine has increased the survival rate of VLBW infants. To improve the long-term developmental outcome of surviving VLBW infants, it is necessary to exclude hypothyroidism and this should be treated appropriately. A newborn mass-screening system measuring TSH (and FT4) is useful to detect primary hypothyroidism.

However, in cases with low levels of F(T4) and normal or low levels of TSH, the current mass-screening system using only TSH (and FT4) is not accurate enough to distinguish THOP from central hypothyroidism.

In this study, we measured TSH levels (0, 30, 60, 90, 120, and 180 min) after the administration of 7 μ g/kg TRH to clarify the pathophysiology of THOP, including an evaluation of hypothalamus function. In the THOP group, the peak TSH values were reached at 30 min after TRH administration and were not significantly different from those of the Non-THOP group; these results were the same as in our previous study. On the other hand, the decrease in TSH after the peak was clearly delayed in the THOP group.

Van Tijin et al. reported that abnormal results of TRH stimulation tests, characterized by delayed timing of the peak TSH elevation and delayed decrease in the TSH after the peak, were seen in cases with congenital hypothyroidism due to TRH deficiency²⁷. Our results were similar to the cases reported by Van Tijin et al. This coincidence suggested the HPT axis in infants with THOP resembled TRH deficiency. Considering that the hypothalamus reaches maturity around GA 26 weeks morphologically, but functionally only shortly before term date³⁴, immaturity of the hypothalamus may be the main contributor to the low secretion of TRH in infants with THOP.

Moreover, in the THOP group, GA, BW, birth length, the rate of mechanical ventilation, and the volume of enteral feeding were significantly different. VLBW infants in the THOP group were immature and clinically sick compared to the Non-THOP group, and we considered that

non-thyroidal illness syndrome (NTIS) may have affected the thyroidal function of VLBW infants with THOP.

NTIS is characterized by serum concentrations of T3 that decrease to low levels without giving rise to an elevated TSH concentration. As the severity and length of the illness increases, there is a fall in both T3 and T4³⁵. The pathophysiology includes suppression of TRH release, reduced T3, T4 turnover, a reduction in liver generation of T3, and increased formation of rT3. NTIS is considered to be an adaptive response to counteract catabolism during fasting and illness. Downregulation of TRH neurons and TSH production impairs negative feedback regulation during illness. Cytokines (IL-1, IL-6, IFN γ , and TNF α), released during illness are considered a major determinant of NTIS³⁶. Deiodinases and peripheral thyroid hormone metabolism are also affected. Illness reduces activity of type 1 deiodinase (D1) and increases activity of type 2 deiodinase (D2) and type 3 deiodinase (D3), resulting in increased rT3^{35,36}.

In our study, T3 levels were lower in the THOP group than in the Non-THOP group, but the difference was not significant. An elevation in rT3 values is commonly seen in NTIS, whereas rT3 levels in the current THOP group were significantly lower than those of the Non-THOP group. Decreased levels of rT3 may have been caused by extremely low levels of T4 production caused by the suppression of TRH secretion at the level of the hypothalamus. These results suggest that peripheral inactivation of T4 was not a major cause of THOP, which is one of the main causes of NTIS.

This study has some limitations. The major limitation was the small number of subjects. We could not recruit enough VLBW infants with THOP to accurately analyze the relationship between the factors of immaturity and the abnormal TRH stimulation test results. The second limitation is that we could not obtain accurate data on maternal and neonatal iodine exposure. In Japan, iodine deficiency is rare because of the custom of eating seaweed containing iodine; however, excess

iodine may be problematic. The third limitation is that we did not measure TBG or cytokines that are considered to affect TRH secretion.

Despite these limitations, the 180 min TRH stimulation test results for VLBW infants are valuable to clarify the pathophysiology of THOP and add new insights to our previous studies.

In conclusion, the delayed decrease in the TSH concentration after the peak on the TRH tests suggests that the main etiology of THOP is suppression at the level of the hypothalamus. Based on a subject's profile, immaturity of the HPT axis, especially at the level of the hypothalamus rather than NTIS, may have a greater impact on the suppression of TRH secretion.

However, additional studies are necessary to clarify these results and confirm the mechanism that leads to the reduction in rT3 in THOP. We need to analyze a much larger population and collect accurate data on maternal and neonatal iodine exposure, TBG levels, and cytokine profiles that affect TRH secretion.

Conflict of Interest

The authors have no conflicts of interest to declare.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figure Legends

Figure 1) Flowchart of the subjects

A total of 47 subjects were born and admitted to the NICU. One subject with severe hypoxic-ischemic encephalopathy (HIE) and three subjects with elevated TSH ≥ 15 mU/L and administered LT4 were excluded. Finally, 43 subjects were recruited for this study.

Figure 2) Correlation between the paper filter spot TSH method and ECLIA method

Serum concentration of TSH 0 min measured by the paper filter spot TSH method was compared with TSH 0 min measured by the ECLIA method. Linear regression analysis showed a good correlation ($r = 0.79$, $p < 0.0001$).

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Table 1. Subject profiles and a comparison between Non-THOP and THOP cases

	Total No.	Non-THOP	THOP
	(N=43)	(N=32)	(N=11)
	<i>p</i> value		
GA, median (IQR), weeks	27.9 (26.4-29.3)	28.3 (27.3-30.1)	26.3 (25.1-27.4)
	<0.01		
BW, median (IQR), grams	990 (774-1174)	1089 (853-1235)	802 (672-892)
	<0.01		
BW SDS, median (IQR)	-1.0 (-1.7 – 0.03)	-1.0 (-1.8 – 0.1)	-0.8 (-0.6 – 0.4)
	0.54		
Birth length, median (IQR), cm	35.4 (32.3-37.5)	36.0 (33.1-38.0)	32.4 (29.0-34.5)
	<0.01		
Birth length SDS, median (IQR)	-0.9 (-1.9 – 0.03)	-1.0 (-2.0 – 0.02)	-0.8 (-1.8 – 0.4)
	0.60		
Gender, n (Male %)	24 (55.8)	17 (53.1)	7 (63.6)
	0.54		
Cesarean Section, n (%)	33 (76.7)	25 (78.1)	8 (72.7)
	0.71		
Apgar at 1 minute, median (IQR)	3.9 (2 – 6)	4.1 (2 – 6)	3.5 (2 – 5)
	0.44		
Apgar at 5 minutes, median (IQR)	6.6 (6 – 8)	6.6 (6 – 8)	6.4 (6 – 8)
	0.70		
Antenatal glucocorticoid, n (%)	27 (62.8)	20 (62.5)	7 (63.6)
	0.95		
RDS, n (%)	29 (67.4)	19 (59.4)	10 (90.9)

	0.051			
Intrauterine infection, n (%)	11 (25.6)	7 (21.9)	4 (36.4)	
	0.43			
Postnatal glucocorticoid, n (%)	16 (37.2)	10 (31.3)	6 (54.6)	
	0.28			
Catecholamine use, n (%)	9 (20.9)	6 (18.8)	3 (27.3)	
	0.67			
Mechanical ventilation at TRH test, n (%)	24 (55.8)	13 (40.6)	11 (100)	
	<0.01			
Enteral feeding at TRH test,	89.5 (31.5-89.5)	107.0 (77.3-137.0)	29.0 (15.0-91.0)	
	<0.01			
median (IQR), ml/kg/day				

GA; Gestational Age, IQR; Interquartile Range, RDS; Respiratory Distress Syndrome

Table 2. TRH stimulation test results

	Total No. (N=43)	Non-THOP (N=32)	THOP (N=11)	<i>p</i>
value				
Postnatal days at TRH test, median (IQR), days	14 (13-14)	14 (13-14)	14 (11-14)	0.27
Corrected age at TRH test, median (IQR), weeks	29.9 (28.3-31.3)	30.3 (29.2-32.0)	28.0 (27.1-29.0)	
	<0.01			
Basal FT4, median (IQR), ng/dL	1.2 (0.8-1.6)	1.5 (1.1-1.7)	0.7 (0.6-0.8)	
	<0.01			
Basal FT3, median (IQR), ng/dL	1.9 (1.3-2.3)	2.1 (1.9-2.4)	1.0 (0.8-1.1)	
	<0.01			
TSH, median (IQR), mU/L				
0 min	6.3 (4.2-8.5)	7.1 (4.9-9.3)	4.2 (2.6-7.5)	0.06
30 min	22.3 (16.8-34.1)	21.7 (15.8-33.3)	23.5 (22.6-40.2)	0.37
60 min	18.5 (15.4-28.1)	17.6 (11.4-24.5)	22.9 (18.5-34.3)	0.08
90 min	15.8 (12.5-25.5)	14.4 (11.4-22.1)	22.2 (15.8-30.2)	

<0.05			
120 min	13.2 (10.1-18.7)	11.3 (9.5-15.9)	18.7 (12.1-18.7)
<0.05			
180 min	9.5 (6.8-13.4)	8.6 (6.5-11.0)	14.1 (9.2-16.4)
<0.05			
180 min / 0 min ratio	1.4 (1.1-2.1)	1.3 (1.0-1.7)	3.0 (1.5-5.3)
<0.01			

Table 3. Thyroid hormone profiles (N=26) and a comparison between those of the Non-THOP and THOP groups

	Total No. (N=26)	Non-THOP (N=20)	THOP (N=6)	<i>p</i> value
GA, median (IQR), weeks	27.6 (26.1-29.0)	28.1 (27.2-29.9)	26.0 (25.0-27.6)	<0.05
BW, median (IQR), grams	891 (772-1149)	1053 (813-1216)	790 (644-853)	<0.05
Antenatal glucocorticoid, n (%)	13 (50.0)	10 (50.0)	3 (50.0)	1.00
RDS, n (%)	19 (73.1)	14 (70.0)	5 (83.3)	1.00
Postnatal glucocorticoid, n (%)	10 (38.5)	7 (35.0)	3 (50.0)	0.64
Catecholamine, n (%)	6 (23.1)	4 (20.0)	2 (33.3)	0.60
Mechanical ventilation, at TRH test, n (%)	15 (57.7)	9 (45.0)	6 (100)	<0.05
Basal FT4, median (IQR), ng/dL	1.2 (0.9-1.6)	1.3 (1.1-1.6)	0.6 (0.6-0.8)	<0.01
Basal FT3, median (IQR), ng/dL	1.9 (1.4-2.2)	2.1 (1.9-2.3)	1.0 (0.7-1.2)	<0.01
T3, median (IQR), ng/mL	1.0 (0.7-1.3)	1.0 (0.8-1.3)	0.7 (0.4-0.7)	0.06
rT3, median (IQR), pg/mL	152.3 (116.8-185.6)	168.0 (148.1-197.0)	92.9 (74.7-101.6)	<0.01
rT3 / T3 ratio, median (IQR)	171.3 (134.2-217.8)	180.2 (136.0-223.7)	139.3 (74.2-222.6)	0.21
rT3 / FT4 ratio, median (IQR)	126.9 (113.9-154.4)	130.5 (113.2-152.4)	120.1 (108.5-173.2)	0.98



