The influence of preanalytical factors on the clinical validity of some endocrine parameters measured with immunoanalytical methods

Ph.D thesis

Dr. Zoltán Lőcsei

Doctoral School of Clinical Medicine,

Semmelweis University

Consultants: Dr. Károly Rácz, professor, D.Sc

Dr. Erzsébet Toldy, associate professor, Ph.D.

Opponents: Dr. István Takács, associate professor, Ph.D.

Dr. Tamás Kőszegi, associate professor, Ph.D.

Head of the Final Examination Committee:

Dr. Péter Lakatos, professor, D.Sc

Members of the Final Examination Committee:

Dr. Barna Vásárhelyi, professor, D.Sc

Dr. Sándor Alföldi, head physician, Ph.D.

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I/ Introduction

Laboratory investigations are fundamental in the diagnostics of endocrine diseases. The success of the endocrine practice is highly dependent on the accuracy of laboratory measurements, because tiny changes in hormonal levels are more specific and sensitive in the diagnostics and in the assessment of therapy effects than the anamnestic data or the physical signs. At the same time laboratory evidence cannot only be important but also misleading. If it offers false data, inaccurate decisions can be made.

Because of the characteristics of immunoassays (matrix effect, antibody cross-reactions, specificity, and sensitivity depending on techniques) the laboratory evidence can sometimes lead to misconclusions, diagnostic errors and disturbances in the follow-up therapy. Sometimes the result mirrors not only the level of the molecules that are wished to be detected, but also the serum environment too. The knowledge of those facts is extraordinarily important concerning circumstances, where laboratory data can be decisive with regard to the fate of the patients or concerning expensive and burdening examinations.

This work examined six important markers (thyroglobulin (Tg), thyroglobulin antibody (TgAb), plazma renin activity (PRA), quantitative renin (REN), aldosteron/renin quotient (ARR: ALD/PRA and ALD/REN)) with the intention of increasing the reliability of laboratory diagnostics in case of differentiated thyroid cancer (DTC) and primer aldosteronism.

1.1.: DTC patients are in need of lifelong care and the early diagnosis of recurrence or metastasis is fundamental concerning the fate of the patients. Tg levels in the blood have no importance at the start of diagnostics, but have an essential role during the follow-up. After total thyroidectomy and I-131 ablation rising Tg levels are able to show the recurrence early, if the sensitivity of the applied method makes the secure measurement of very low levels possible. Guidelines are consonant in the fact, that Tg measurement is essential in case of the care of DTC patients. At the same time the applied immunanalytical method for Tg determination makes reliability of the results questionable. The measurement of Tg is irrational without the knowledge of TgAb levels. If the measuered Tg level with TSH stimulation is less than 1 ng/ml, we can consider the patient to be in a complete remission, and we do not perform whole body scans or other imaging investigations, except ultrasound scan. At the same time rising Tg levels could indicate a new I-131 therapy without any other cases of tumour recurrence. The decisions of fundamental importance for the patient are made on the basis of Tg levels and the aforementioned factors which may cause concern with regard to the accuracy of measurements and may thus call for further investigation This is considered one of the objectives of this work.

1.2: Conn described in 1954 for the first time the consequences of the ALD overproduction due to an adrenal adenoma. The simultaneous symptoms of hypertension, hypopotassemia and metabolic alkalosis have been named as **Conn syndrome. (PA**). Recent estimations made clear,

that in 5-13% of all hypertonic patients PA can be held responsible for the elevation of the blood pressure. According to statistical data in 2003 in Hungary there were 211 hypertensive patients per 1000 inhabitants. So we can estimate that there are more than 210000 patients with PA in Hungary, but in practice we diagnose only a fraction of them. The importance of the recognition of PA can be explained not only from the viewpoint of the frequency of PA, but also from the fact, that the cardiovascular mortality of PA patients is much higher compared with patients of the same gender, age and blood pressure levels. The medical treatment of PA patients is different from the conventional treatment of hypertensive patients. Guidelines recommend PA screening as the first step in the estimation of ARR. Less investigated territory nowadays concerns the effect of female hormone products on the ALD and renin levels, though in practice we can encounter not infrequently, hypertensive patients taking oral anti-concipients(OC) for a long period of time. Determination of renin concentrations requires very strong sampling and pre-analytical conditions. Traditionally renin determination is made with the use of PRA measurement, but in recent years the REN determination has become widely accepted. The precise determination of ARR has great importance in the diagnostics of PA, but we can only expect reliable diagnostic values in the case of precisely defined pre-analytical circumstances, weighting clinical conditions, medical treatment and method specific cut-off values. In the absence of the above-mentioned factors we might generate expensive and burdening investigations (in case of falsely high ARR) or we can delay the diagnosis of PA (in case of falsely low ARR). The investigation of determinant factors in this field is considered the other main objective of this thesis.

II. **Aims**:

II.1. The investigation some in vivo and in vitro preanalytical factors influencing Tg and TgAb determinations

- To investigate the influence of the storage conditions of serum samples (cooling, time interval until measurement) on the accuracy and reliability of determinations.
- The sensitivity analysis of Tg determination on TgAb between in vitro circumstances primarily by lower antibody titers. Is it possible, that lower TgAb titers have importance in Tg measurement in the care of DTC patients?

II.2 The investigation of essential markers of PA diagnostics:

- To investigate the preanalytical factors concerning the measured PRA, REN and ALD levels and the calculated ARR values.
- In what way do the management and storage methods of blood samples influence the two different renin estimations (adherence to the exact sampling protocol, cooling, time interval until measurement)

- The analysis of the reliability of the measurement of the above-mentioned molecules in the circumstances of routine outpatient practice of sampling and transport methods (different transport and storage times, thermal conditions). Taking into consideration these circumstances it is important to find out what alters the ARR value?

- The investigation of the clinical validity of the two methods in hypertensive patients in case of common practice in internal medicine.

    In hypertensive patients without any medical treatment, and in patients taking beta blockers, Ca-antagonists or ACE blockers.

    In patients with incidental adrenal adenomas

- The determination of decision making in the case of cut-off values of ARR from the results of healthy volunteers with normal tension and its comparison with the well-defined values of international guidelines.

- The investigation of the influence of oral contraceptives (OC) with altering gestagene component on the measured aldosterone and renin levels.

    The impact of gestagene components with altering pharmacological effect (gestodene: GTD, desogestrel: DSG, drospirenone: DRSP) on the measured ALD, PRA and REN levels

    In what way do the OC with altering gestagene and ethylestradiol component influence the ARR values compared with the data of healthy volunteers taking no pills.

    The change of decision making in the case of cut-off values of ARR compared with healthy, normotensive women in reproductive ages taking or not taking OC.

III: **Material and methods**

III.1: Investigated persons and serum samples for the pre-analytical studies

In the in vitro experiments 161 individual or pooled serum samples were used ((Tg-TgAb: 135; PRA-REN: 36) for the analysis of pre-analytical conditions. During the course of these experiments altogether 954 measurements from 399 samples were examined. There were 149 healthy volunteers for the PRA-REN investigations who represented the control groups. The number of serum samples from 112 patients with well defined illness ((Tg-TgAb 27, PRA-REN 85) was 466 with controls, that meant 1146 laboratory determinations for the parameters.

III.1.1 The investigation of the stability of Tg and Tgb molecules and the functional sensitivity of the method

Stability investigations: 10 samples were used for the short term, and 7 samples for the long term storage investigations. In both the time intervals of re-measurements were chosen from zero hour to simulate the routine working process of the laboratory.

Determination of functional sensitivity: For this purpose, serum samples were investigated with Tg and TgAb content near to the analytical sensitivity and to the low cut-off level of decision making of the method. In case of Tg 7 samples with variant Tg concentration (minimal 0,24 maximal 14,4 ng/ml) and with TgAb level less than 20 IU/ml were used. For the electrochemoluminescent TgAb method (ECLIA Roche) 8 serum pool with variant TgAb titer (minimal 16 maximal 150 IU/ml) were used. The functional sensitivity of the chemoluminometric TgAb method ((CLMA, Abbott) was determined in 7 samples with different TgAb level ((minimal: 0,36; maximal: 1,06 IU/ml)

III.1.2 The influence of TgAb titers on the measured Tg levels in vivo and in vitro.

In vitro experiments: In a three-year period a TgAb manipulating experiment took place on two occasions with several measurement series. In the first experiment only one kind of TgAb calibrator was used (Roche). For the second two different TgAb methods (ECLIA, Roche; CLMA, Abbott) with three different TgAb calibrators, one sheep (Roche) and two human (Roche and Abbott) were used for in vitro manipulation. We added an increasing amount of TgAb calibrator to serum pools with different Tg level with the purpose of approaching or surpassing the decision level of antibody positivness (115 IU/ml). 28 serum pools either with indetectable or under the functional sensitivity TgAb level were investigated with two methods.

III.1.2.2 Adaptation of the in vitro results on the Tg levels of clinical cases.

In the care of 27 DTC patients (22 women, 5 men, mean age 47±13 years, median value of follow time 2,5 (0,5-8,0) years) 134 serum samples were obtained and analyzed. The number of samples obtained from the same patient was between 2 and 6. Tg and TgAb levels were measured in all samples. From these data and from the data of the connection experienced in vitro between Tg and TgAb calculated Tg levels were estimated when the TgAb level was above the detectable limit (24 IU/ml) in the patient sample.

III.1.3 The effect of sampling and storage conditions on PRA and REN levels

III.1.3.1: Investigation of sampling conditions: Blood was drawn from 26 healthy volunteers within 2 hours after getting up between 8 and 10 o'clock a.m. in undisturbed sitting position. 86 samples were obtained of which pre-analytical conditions were carefully established prior to the freezing. The identically handled samples formed 6 groups.

III.1.3.2: Investigation of storage frozen for a longer period of time. Samples were donated by 10 healthy volunteers. Handling was precisely in accordance with the protocol concerning REN and PRA defined by the manufacturer. The values measured from the fresh, centrifuged plasma were

considered as 0 (starting point) level. After that each plasma was divided into 3 portions and was stored in plastic tubes for 2, 5 and. 7 weeks at -20 Cº.

III.1.4 Comparison of ARR values calculated from PRA or REN levels.

III.1.4.1. Samples from medical patients: Samples were obtained from 134 individuals. (80 women, 54 men, mean age 46,1±15,5 years). 49 healthy normotensive volunteers served as a control group. 29 patients with incidentally recognized adrenal adenomas (among them 19 hypertensive) and 56 hypertonic patients (among them 34 untreated, 25 treated) were investigated too. Among the latter patients 9 patients were on ACE or ARB blocker, 9 patients were on beta blocker and 7 patients were on dihydropirine type Ca-channel antagonist treatment

III.1.4.2: The effect of OC with altering gestagene component on the PRA, REN and ARR values.

Sampling took place involving 86 healthy normotensive women with normal potassium level. Mean age was 27,3±7,5 years. Among them 63 patients were taking OC for a longer period of time (2,8±2,3 years) with different gestagene components (25 GTD, 22 DSG, 6 DRSP). Each pill also contained low amount of etenilestradiol (0,02-0,035 mg) .

IV Results

IV.1. The investigation of the stability of Tg and Tgb molecules and the functional sensitivity of the method in different pre-analytical circumstances.

IV.1.1: Short term storage also has a significant influence on the measured Tg levels. The storage of the samples at room temperature made a difference only to a small degree (5-7%), but in the case of storing at 4-10 Cº the Tg immune reactivity grew gradually. After 24 hours the difference surpassed the value of the variational coefficient of the method (CV=8%), and after 48 hours it attained 23%.

IV.1.2. In case of long term storage at -17-20 ºC the Tg and TgAb levels decreased significantly. (Tg: $p<0,0001$, TgAb: $p<0,01$) The decrease above the CV of the method took place on the 3rd week in the case of Tg and on the 2nd week in the case of TgAb.

IV.1.3. The influence of TgAb titers on the measured Tg values. There was a weak but significant correlation ($r= -0,38$) between Tg recovery (Tg%) and TgAb titers. At linear regression analysis between TgAb and the measured Tg levels there was a demonstrable correlation with negative steepness (-1,76) and with strong determinant coefficient. ($R^2=0,74$) It is possible to interpolate a power function with strong correlation coefficient on the variables ($r=0.97$; $p<0,001$). This fact establishes the possibility of elaborating a mathematical relation for the methods. If we know the Tg and TgAb level of a given sample, we are able to calculate the decrease of Tg level caused by TgAb. Human antibody showed a significantly lesser Tg loss with the same TgAb increase, as the sheep antibody did.

IV.1.4 **The analysis of the samples of DTC patients with consideration of in vitro results**. In 134 samples of DTC patients divided into groups on the basis of TgAb titers, measurable but not elevated (24-115 IU/ml) titer was found in 79% (N=106) of the patients. It was possible to demonstrate a significantly ($p<0,02$) higher TgAb titer in the sera of patients with undetectable Tg levels in comparison with the samples with detectable Tg level. Applying the equation gained from the in vitro studies, we were able to calculate Tg values from Tg and TgAb levels, and these values were significantly higher than the measured ones.

IV.2.1. **The influence of sampling and storage conditions on the measured PRA and REN levels:** After storing the samples in room temperature, PRA, as well as REN levels decreased significantly ($p<0,001$), but they stayed unchanged, when the samples were stored at 0-5 Cº. REN values were significantly elevated when sampling took place in pre-chilled tubes, and the storage took place at 0-5 Cº between 30 minutes as compared to the samples stored at room temperature. When storing at -20 C, PRA decreased at 9.4±2,4%. The decrease is statistically significant but not relevant taking the CV intra-assay values of the method into consideration. The decrease of PRA was more relevant on week 5 (15.2±4.7%; $p<0.02$), and on week 7 (31.9±4.7%; $p<0.01$) compared to the beginning. Contrary to this, the decrease of REN investigated at the same time intervals was not significant, remaining under 5%.

IV.2.**2 Comparison of ARR values gained by two different renin methods**. The decision making cut-off levels for PA diagnostics were determined at 97,5 percentage by 30 ng/dl/ng/ml/h in case of PRA and by 3 ng/dl/ng/l in case of REN.

IV.2.**3 Correlation between the two different renin methods:** Between PRA (1.2±14 ng/ml/h) and REN (12,1±11,0 ng/ml) a significant strong positive correlation ($r=0.84$) was observed in the case of all the investigated samples (n=134). In the range lower than 1.5 ng/ml/h (n=103, PRA: 0,63±0,41 ng/ml/ h, REN: 8.1±4,9 ng/ml) the correlation was weaker ($r=0.59$), but similarly significant ($p<0.001$).

IV.2.4: Clinical experiences with the two different methods

IV.2.4.1. Untreated patients Compared to the groups without antihypertensive medication using median test only, ALD levels altered significantly ($p<0.01$). At the same time investigating with ANOVA test after rang transformation; significant difference was not observed between the groups only in the case of REN. In PA group –as expected- the ratios calculated with both methods were the highest, and differed significantly from all the other groups. The ALD levels in the normotensive group with adrenal adenoma were significantly lower compared to the control patients and hypertensive patients.

IV.2.4.2 Treated patients: There was a significant difference in case of PRA ($p<0.001$) and in case of REN ($p<0.05$) using median test. However, investigating with ANOVA test after rang transformation only ALD levels altered significantly. The most meaningful difference was observed in the case of PRA and ALD/PRA ($p<0.01$). The ALD/REN ratio in the PA group was

significantly higher compared to the untreated hypertensive patients and with the patients treated with ACE/ARB or Ca-channel antagonists.

Comparing the control group and the untreated patients with the group of treated patients a significant alteration can be found only for ALD/PRA. In patients receiving beta blocker treatment the ALD/PRA values were significantly higher ($p<0.01$), compared to the control or to the untreated patients, or to patients treated with ACE/ARB or Ca-channel antagonist. In case of ALD/REN such difference could be observed only in the Ca-channel group. ($p<0.001$). The same is true between control patients and the Ca-channel antagonist group concerning ALD/REN. ($p<0.01$).

IV.2.4.3 **The effect of OC with altering gestagene component on the ALD, PRA, REN and ARR values**

The PRA, REN and ALD levels were significantly higher in the DRSP group compared to the DSG, GTD and the control group. [PRA: 3,1 (1,5 3,7) vs. 1,4 (1,1 2,0); 1,2 (0,8 2,2); 1,3 (0,7 1,6) ng/ml/h, REN: 25,2 (9,7 30,3) vs. 8,3 (6,8 12,3) ; 8,0 (4,8 10,5); 12,2 (7,5 21,7) ng/ml, ALD: 43,7 (28,0 61,6) vs 11,5 (7,2 16,6); 13,4 (7,7 22,1); 10,0 (4,4 14,7) ng/dl]. The ratio of ALD/PRA in the DRSP group was significantly ($p<0,01$) the highest [15.4 (11.6 22.6) ng/dl/ng/ml/h] and differed significantly from the control and from the DSG, GTD group. The ALD/PRA ratios in the DSG and the GTD group did not differ significantly from the ratios in the control group. The ALD/REN ratio was significantly the highest in DRSP (2,0 (1,5-2,6) group. When compared to the control group[1,1 (0,4-1,5)] significantly ($p<0,01$) higher values were observed in the GTD [1,6 (1,2-2,4) ng/dl/ng/ml] group too.

V: **Conclusions, new statements**

V.1.1 The investigation of the stability of Tg and Tgb molecules and the functional sensitivity of the method in different pre-analytical circumstances.

It is appropriate to store the samples at 4-10 Cº only for a time interval that is shorter than 24-28 hours for both laboratory methods.

It is appropriate to store the frozen samples at -17-20 Cº only for a time period shorter than 2-3 weeks, even in those cases, when economic interests of the laboratory motivate sparse measurements.

The frozen storage of samples for Tg determination in the long run should be avoided as far as possible, if low levels can be expected (as in most cases of DTC patients)

V.1.2 **The influence of TgAb titers on the measured Tg levels in vivo and in vitro**

Low quantity of TgAb titers,-considered to be in the normal range- have a remarkable influence on Tg measurement. They have the ability to make the Tg, that is present in measurable level, undetectable.

The change in the measured Tg levels caused by increasing TgAb titers can be modeled and described mathematically

It is necessary to take into consideration low TgAb levels in the case of DTC patients. In patients with such levels the non-detectable Tg values do not indicate the low risk with certainty for the patients.

Using corrected Tg levels, calculated on the basis of a mathematical model can be helpful in certain cases in the case of DTC patients

V.2.1 **The influence of sampling and storage conditions on the measured PRA and REN levels**

The storage of the samples at room temperature even for two hours can cause the decrease of the measured levels, in contrast with the storage at 0-5 Cº. The latter method is recommended. In this case there in no need for a centrifuge at the site of the drawing of blood in cases when the sample arrives to the laboratory within two hours. If the separation of plasma can be assured within 30 minutes, it is possible to store the samples at room temperature.

It is advised to use up frozen samples in a two-week period for PRA determination in order to avoid inaccurate low values due to the storage. In laboratories with lower investigation sample numbers, the weekly measurement can be uneconomical. In these places the change-over concerning the REN method is worth being considered.

V.2.2 **Comparison of ARR values obtained with two different renin methods**

There is a noticeable correlation between the two methods (PRA and REN), -with the lower- diagnostically more relevant- however, in case of low values the correlation is weaker.

When investigating the effect of antihypertensive medication the most pronounced deviation was found in the beta-blocker group, while the effect of dihydropridin Ca channel antagonists was not significant. It seems that these medicaments in the latter group are beneficial for the safety of the patients without limiting the diagnostic accuracy of both methods.

When determining cut-off values for our own laboratory in the case of ALD/PRA, we found identical values to the literary data. In the case of ALD/REN the value was slightly lower.

V.2,3 **The effect of OC with altering gestagene component on the measured ALD, PRA, REN and ARR values**

OC-s have an influence on the measured ALD, PRA, REN and ARR values, the degree of influence depends on the gestagene component.

The elevation of the measured values was the most pronounced in the DRSP group.

In women taking OC with DSD or GTD component the PRA determination is favorable for REN determination.

It would be practical to determine cut-off values for women taking OC, taking into consideration the gestagene component of the pills.

VI. Catalogue of publications attached to the thesis

VI.1 In extenso publications:

1. **Lőcsei Z**, Toldy E, Szabolcs I, Rácz K, Kovács G L. (2009): The effect of sample storage on the reliability of thyroglobulin and thyroglobulin antibody measurments Clin Biochem. 42: 225-228 IF: 2,019

2. **Locsei Z**, Rácz K, Patocs A, Kovacs G L.,Toldy E. (2009): Influence of sampling and storage conditions on plasma renin activity and plasma renin concentration. Clinica Chimica Acta 402 203-205 IF: 2,535

3. **Lőcsei Z,** Horváth D, Rácz K, Szabolcs I, Kovács GL, Toldy E. (2012): Progestin-dependent effect of oral contraceptives on plasma aldosterone/renin ratio. Clin. Biochem. 45:(16-17):1516-18 IF: 2,076

4**. Locsei** Z, Szabolcs I, Rácz K, Kovács GL, Horváth D, Toldy E: (2012): Serum thyroglobulin antibody levels within or near to the reference range may interfere with thyroglobulin measurement Biochem Med (Zagreb). 22:365-70. IF: 1,343

5. Toldy E**, Lőcsei** Z, Kovács L G.(2010) A betegektől az analitikáig: az endokrin betegségek felismerését megtévesztő preanalitikai hibákról. Egészség-Akadémia 1: 187-203

6.**Lőcsei Z**, Horváth D, Rácz K, Toldy E. (2011) Szérumtireoglobulin és tireoglobulin-antitest együttes meghatározásának jelentősége a differenciált pajzsmirigy-karcinómás betegek gondozása során. Orv. Hetilap. 152: 743-752

7. Horváth D, **Lőcsei** Z, Csizmadia Zs, Toldy E, Szabolcs I, Rácz K. (2012): A renin-aldoszteron rendszer vizsgálata két módszerrel különböző klinikai állapotokban. Orv Hetilap 153: 1701-1710

VI.2: Book chapters

1.	Kovács L.G, Toldy E, **Lőcsei Z.** (2001) Általános módszertan. Az endokrin betegségek diagnosztikájában használatos laboratóriumi módszerek In: A klinikai endokrinológia és anyagcsere betegségek kézikönyve. Szerk: Leövey András. Medicina Budapest 51-62.
2.	Toldy E, Kovács G L, **Lőcsei Z.** (2001): A leggyakrabban alkalmazott laboratóriumi módszerek ismertetése és értékelése. Az endokrin betegségek diagnosztikájában használatos laboratóriumi módszerek In: A klinikai endokrinológia és anyagcsere betegségek kézikönyve. Szerk.: Leövey András. Medicina, Budapest 63-94.
3.	Kovács L G, Toldy E, **Lőcsei Z**, Mezősi E. (2008) Endokrinológiai laboratóriumi vizsgálatok In: Gyakorlati laboratóriumi medicina. Szerk.: Debreczeni L. és Kovács L. G. Budapest, Literatura Medica 397- 424.
4.	**Lőcsei Z.** Toldy E, Kovács L G. (2008) Laboratóriumi esettanulmányok In: Gyakorlati laboratóriumi medicina. Szerk.: Debreczeni L. és Kovács L. G. Budapest, Literatura Medica 525-528
5.	Toldy E, **Lőcsei Z.** (2011) Endokrin és anyagcsere-vizsgálatok laboratóriumi leleteinek értékelése. In: Az endokrin és anyagcsere betegségek gyakorlati kézikönyve. ed: Leövey A., Nagy V. E., Paragh Gy. Rácz K. Medicina, Budapest 50-56.

VI.3: Citable abstracts

1.	**Lőcsei Z,** Toldy E, Méhes M, Kovács L G. (2006) A tireoglobulin- és tireoglobulin antitest szintek együttes mérésének jelentősége a differenciált pajzsmirigy carcinomás betegek gondozása során Magy Belorv Arch6. 59 Suppl 1: 50.
2.	Toldy E, **Lőcsei Z**, Papp Z, Kovács l G. (2006) Hol van a tireoglobulinantitest-szintek referenciahatára a differenciált pajzsmirigy carcinomás betegekben? Magy Belorv Arch. 59 Suppl.l : 69.p
3.	**Lőcsei Z.** Toldy E, Papp Zs, Kovács L G. (2006) A tireoglobulin és tireoglobulinantitest-szintek együttes mérésének jelentősége a differenciált pajzsmirigy-carcinomás betegek gondozásában Magy Belorv Arch Suppl.4: 86.
4.	Toldy E, Papp Zs, **Lőcsei Z,** Kovács L G. (2006) A thyreoglobulin és thyreoglobulin antitest-szintek együttes mérésének jelentősége a differenciált pajzsmirigy-carcinomás betegek gondozásában Klin Kisérl Lab Med 32. Suppl. 39
5.	Toldy E. **Locsei Z**, Szabolcs I, Kovács GL. (2007) From sampling to analytics: experience and diagnostic consequences with some thyroid markers. Endocrine

	Abstracts. 14: 331.
6.	**Löcsei Z**, Toldy E, Szabolcs I. Kovács LG.(2007) Thyroglobulin antibodies in the normal range decrease the diagnostic accuracy of thyroglobulin in the care of patients with differciated thyroid cancer. Endocrine Abstracts . 14 : 141.
7.	Toldy E, **Löcsei Z**, Szabolcs I. Kovacs LG.(2007) Assay-dependent interference of normal thyroglobulin levels with thyroglobulin measurement. Clin Chem and Lab Med Spec suppl T 379.
8.	Toldy E, **Lőcsei Z** Krkos K. Rácz K. (2008) A primer aldoszteronismus szűrési módszere: a mintavételtől a laboratóriumi analízisig Magy Belorv Arch 3: 265.
9.	Toldy E., **Lőcsei Z.** Catomio Cs, Krkos K. Rácz K. Kovács L G. (2008) A mintavételtől a primer aldoszteronismus szűrésének kezdetéig. Klinikai és Kis. Lab. Med. 33 , 40.
10.	Kovacs LG. Horvath D, Nagy R **Locsei Z**, Toldy E. (2009) Comparison of plasma renin activity with quantitative renin: drug interferencies Clin Chem and Lab Med 47: S 239
11.	**Lőcsei Z.** Horváth D Nagy R. Toldy E. Rácz K. (2010) Helyettesíthető-e a plazma renin aktivitás az aktív renin mérésével? Magy Belorv Arch . 63: 219
12.	**Locsei Z.** Toldy E, Horvath D, Nagy R. Racz K. Szabolcs I, Kovacs LG.(2010) Comparison of plasma aldosteron/signal activity and aldosterone/active signal ratio in different clinical conditions Endocrine Abstracts 22: P61
13.	Horváth D., **Lőcsei Z,** Nagy R, Toldy E, Rácz K, Kovács LG. (2010) Can we replace aldosterone/plasma renin activity ratio with aldosterone/active renin ratio. Laboratóriumi Medicina 35: P15 177