Review

The path toward routine use of LC/MS/MS in clinical chemistry laboratories in Japan

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Abstract Mass spectrometry (MS) has increasingly been used in a wide range of biomedical fields in Japan. Among these applications, the most successful in laboratory medicine is the rapid identification of microorganisms by MALDI-TOF MS, leading to a revolutionary shift in clinical diagnostic microbiology.

In sharp contrast, the adoption of LC/MS-based assays for routine use in clinical chemistry laboratories in Japan has been very slow and limited. Although LC/MS/MS offers several advantages over immunoassays, including higher specificity, multiplexing capability, and independence from antibodies, the adoption of this technology in the real world of laboratory medicine has been hindered.

The major reasons for the limited use of LC/MS/MS in routine laboratory practice in clinical chemistry, despite its great advantages, are: 1) Laboratory tests for outpatients in Japan are expected to be performed as same-day tests, which require a rapid turnaround time within 60 minutes and high throughput, posing a challenge to current LC/MS/MS assay systems. 2) MS-based measurements conducted by laboratory-developed LC/MS/MS assays are not covered by health insurance for the majority of test items for which in-vitro diagnostics are available.

To accelerate the wide adoption of LC/MS/MS in clinical chemistry laboratories, achieving full automation of the LC/MS/MS system (preferably with a push-button design) with bidirectional access to the Hospital Information System is desperately needed. Additionally, resolving the issues related to the reimbursement of laboratory-developed LC/MS/MS testing is mandatory. Furthermore, standardization and harmonization of LC/MS/MS-based methods is another crucial step toward wider acceptance of LC/MS/MS.

Key words: clinical chemistry laboratory, LC/MS/MS, reimbursement, laboratory-developed test (LDT), full-automation

1. Introduction

Mass spectrometry (MS) has been increasingly used in a wide range of biomedical fields in Japan, as outlined in Table 1. Among these applications, the most successful in laboratory medicine is the rapid identification of microorganisms by MALDI-TOF MS, leading to a revolutionary shift in clinical diagnostic microbiology, as reviewed elsewhere[1−3].

In sharp contrast to the highly successful applications of MALDI-TOF MS in clinical microbiology, the adoption of LC/MS-based assays for routine testing in clinical chemistry laboratories has been hindered particularly in Japan.

In this review, we first summarize the significant advantages of LC/MS/MS over conventional methods such as immunoassays. We then discuss why the adoption of LC/MS in routine clinical chemistry has been limited and slow in Japan, despite its great advantages. Additionally, we will discuss what is needed to accelerate the routine adoption of LC/MS/MS technologies in clinical chemistry in Japan.

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2. The current distribution of test methodologies used routinely in clinical chemistry laboratories

The distribution of test methodologies used in the clinical chemistry laboratories at Chiba University Hospital over the past year (2023) is shown in Fig. 1. Each individual test item, such as aspartate aminotransferase (AST) and LDL-cholesterol, was counted as one test. There were a total of 5,311,088 tests. The vast majority of these tests were conducted using colorimetry and immunoassays, performed with fully automated analyzers. Although MALDI-TOF MS has been routinely employed in clinical microbiology at Chiba University Hospital since 2010, the application of LC/MS/MS remains very limited.

3. The advantages of LC/MS/MS over immunoassays

There are many advantages of LC/MS/MS compared to immunoassays, as listed in Table 2. LC/MS/MS offers high specificity, multiplexing capability, independence from antibody availability, and may be advantageous when submitting manuscripts to scientific journals.

Table 1. Representative Usage of Medical Mass Spectrometry in Japan

- Nationwide and publicly funded expanded newborn screening by tandem mass spectrometry
- LC/MS/MS and/or GC/MS as tools for identifying the cause of death or crime in forensic medicine and toxicology
- LC/MS/MS for therapeutic drug monitoring
- Rapid identification of microorganisms by MALDI-TOF MS
- Determination of test items in clinical chemistry for which in-vitro diagnostics are not available
- Gold standard when developing in-vitro diagnostics based on conventional technologies

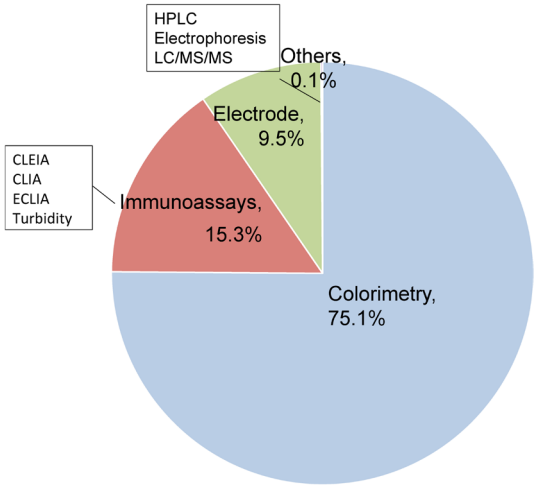

Fig. 1. The Distribution of Test Methodologies used in the Clin Chem Labs at Chiba Univ Hospital in 2023 (A total of 5,311,088 tests).

3.1. Higher specificity compared to immunoassays

It is well known that MS is more specific than immunoassays. I will present an example based on our recent experiences.

In patients with estrogen receptor-positive breast cancer, the menopausal state must be accurately assessed by measuring serum estradiol levels to determine treatment strategies. Due to the structural similarity between fulvestrant (a selective estrogen receptor degrader) and β-estradiol, fulvestrant can interfere with immunoassays of estradiol, resulting in falsely elevated estradiol levels.

During the one-month period of 2019, estradiol measurements for a total of 153 consecutive specimens were requested from the Division of Breast Surgery at Chiba University Hospital. These specimens were measured by immunoassay (ARCHITECT Estradiol II kit; Abbott, Chiba, Japan) and by the LC/MS/MS method we previously reported[4]. Although there was an overall rough correlation between the values obtained by the two methods, it was noteworthy that extremely high values were obtained by the immunoassay in the five specimens obtained from subjects receiving fulvestrant (Fig. 2). It is likely that fulvestrant interfered with the antibody-based estradiol assays. These overestimations could impact treatment decisions. It is clear that LC/MS/MS is preferable in this setting[5].

Immunoassays continue to advance. Measurement of serum thyroglobulin (Tg) is a good example. Serum Tg levels are used for monitoring post-surgery patients with thyroid carcinoma. Anti-Tg antibodies (TgAb), present in about 25% of patients with thyroid carcinoma, can interfere with conventional antibody-based Tg measurements, leading to falsely low Tg values. Therefore, LC-MS/MS, which

Table 2. The Advantages of LC/MS/MS Over Immunoassays

- Higher specificity
- Multiplexing capability
- Smaller sample volume required for each test item due to multiplexing
- Lower running cost
- Independence from antibody availability
- Advantageous when submitting manuscripts to scientific journals

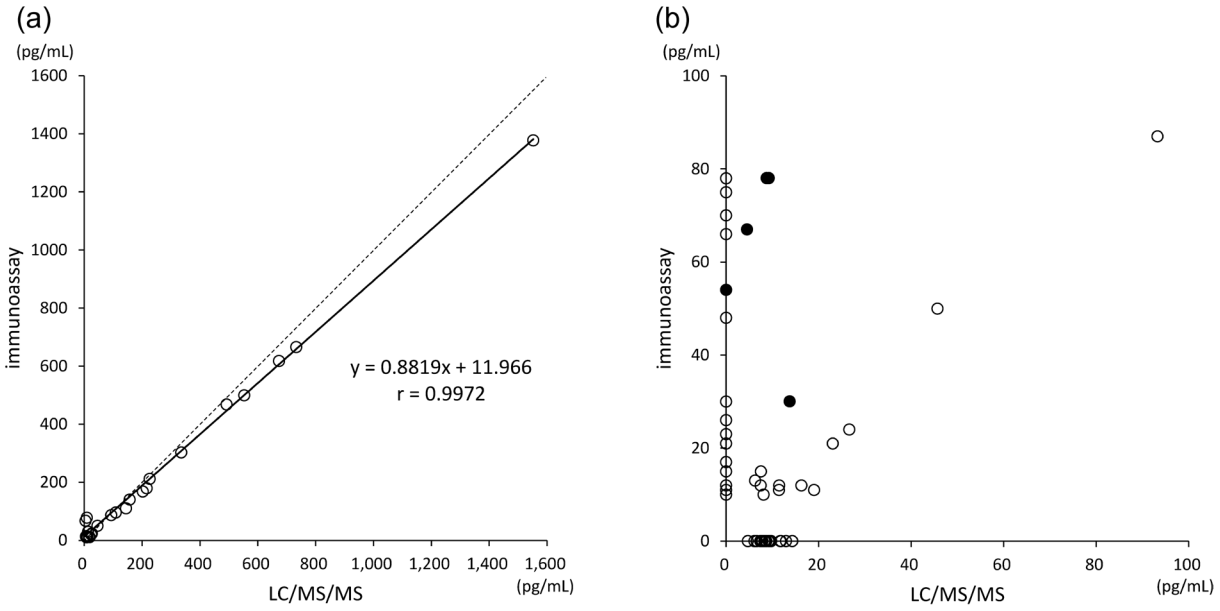

Fig. 2. Linear regression analysis of serum 17-β estradiol levels determined by commercial immunoassay (IA) and LC/MS/MS (MS) on a total of 158 specimens obtained from patients with breast cancer.

Since most of the patients were post-menopausal, the values in 91 specimens were below the LLOQ of both IA and MS. Data for both IA and MS were obtained in 28 specimens. The correlation in these 28 samples is presented in Fig. 2(a). An expanded view of the low concentration range in (a) is presented in (b). The closed circles indicate the 5 specimens obtained from patients receiving fulvestrant.

is free from TgAb interference, has been strongly recommended as the first choice for post-surgery Tg monitoring in Western countries[6].

Recently, a Japanese corporation developed a thyroglobulin immunoassay that can provide accurate Tg levels even in the presence of TgAb[7]. Indeed, the results obtained by this novel immunoassay in serum specimens containing TgAb agreed well with those obtained by LC-MS/MS[8]. Thus, it is likely that the most updated and sophisticated Tg immunoassay has analytical performance comparable to that of LC-MS/MS. It is important to note that a fair comparison must be made between LC-MS/MS and the most updated and sophisticated immunoassay before concluding that LC/MS/MS is preferable.

3.2. Multiplexing capability

A unique advantage of LC/MS/MS is its ability to simultaneously measure multiple analytes. Vitamin D is a representative target. LC/MS/MS allows for the simultaneous measurement of 25(OH)D_3, 25(OH)D_2, 3-epi-25(OH)D_3, and 24,25$(OH)_2D_3$, as we reported elsewhere[9]. In collaboration with our group, Takatani et al. recently demonstrated that the 3-epi-25(OH)D_3 to 25(OH)D_3 ratio in pregnant women is significantly associated with neonatal birthweight, independent of 25(OH)D_3 levels[10]. This suggests that accurate and simultaneous measurement of vitamin D metabolites by LC/MS/MS could contribute to a better understanding of the effects of vitamin D status on birth outcomes.

3.3. Independence from antibody availability

Antibody-based immunoassays are entirely dependent on the availability of specific antibodies. For instance, the measurement of plasma arginine vasopressin (AVP) is crucial for the differential diagnosis of polyuria. However, in 2013, an unexpected event occurred in Japan: the radioimmunoassay (RIA) kit used for AVP measurement became unavailable due to the sudden discontinuation of the antibody supply. As a result, the measurement of plasma AVP at major commercial laboratories in Japan was abruptly and completely halted for several months until a new RIA kit was approved and became accessible[11].

As long as we rely on antibodies, similar disruptions can occur. Therefore, we developed an LC/MS-based method that does not depend on antibody supply for determining human plasma AVP[12]. The lower limit of quantification (LLOQ) of the LC/MS/MS for plasma AVP was lower than that of RIA. Additionally, the turnaround time for the MS method was approximately 60 min, significantly shorter than that of the RIA currently available in Japan. We are

currently working to promote this MS-based method in the clinical community in collaboration with endocrinologists specializing in pituitary diseases. However, making this laboratory-developed test (LDT) reimbursable in Japan is a challenging task, as discussed below.

Primary aldosteronism (PA) is a major cause of secondary hypertension. Accurate measurement of plasma aldosterone concentration (PAC) is essential for diagnosing PA. Conventionally, PAC was measured by radioimmunoassay (RIA) in Japan, with a cut-off level for PA screening set at 120 pg/mL. However, due to the discontinuation of the conventional RIA assay, novel enzyme immunoassays were developed, utilizing the LC/MS/MS-based method as the gold standard[13].

The plasma aldosterone concentration (PAC) obtained by the novel CLEIA method agreed well with those measured by LC/MS/MS[13]. However, results obtained by the conventional RIA were approximately twice as high as those from LC/MS/MS[13].

The previous cut-off level of 120 pg/mL obtained by RIA is approximately equivalent to 60 pg/mL obtained by the novel CLEIA.

Based on these findings, the cut-off level for PAC in screening for primary aldosteronism was revised from 120 to 60 pg/mL in the new clinical practice guidelines for primary aldosteronism[14]. This change caused confusion among clinicians. Ideally, it would be preferable to determine PAC by LC/MS/MS routinely, but we are not yet prepared to implement this in Japan.

3.4. Advantageous when submitting a manuscript to scientific journals

In the editorial of the Journal of Clinical Endocrinology and Metabolism (JCEM) in 2013, it was announced that sex steroid assays must be conducted using MS-based methods for a manuscript to be submitted to the JCEM[15]. This policy was initially intended to take effect in 2015. However, this provocative statement sparked significant controversy, leading to the formation of a sex steroid assays reporting task force within the academic community.

In the latest JCEM author guidelines[16], it is not explicitly stated that LC/MS/MS is mandatory. Instead, specific examples of issues, such as the measurement of sex steroids and vitamin D, are described, implying that LC/MS/MS is preferred. Thus, LC/MS/MS is recognized as a highly accurate and promising technology. However, it should be noted that there are various pitfalls and challenges, such as ion suppression resulting from matrix effects[17].

4. Reasons limiting the routine use of LC/MS in clinical chemistry in Japan

Despite the numerous advantages of LC/MS/MS over conventional assays, the adoption of LC/MS in clinical chemistry has been very limited and slow in Japan. In our opinion, the reasons for this can be summarized as shown in Table 3.

4.1. Lack of recognition for the advantages of MS-based assays

Firstly, the advantages of MS-based assays over immunoassays, as mentioned earlier, are not well recognized in the clinical community, including among medical and technical personnel in laboratory medicine. Specialists in clinical mass spectrometry need to communicate the substantial benefits of MS over immunoassays to the medical community. I believe that certified medical mass spectrometrists, recognized by the medical mass spectrometrists certification program[18] of the Japanese Society for Biomedical Mass Spectrometry (JSBMS) (with 370 certified as of June 2024), can play a significant role in conveying these messages.

4.2. Laboratory tests for outpatients must be performed as same-day tests

Secondly, in Japan, the vast majority (>99%) of laboratory tests for outpatients are performed as same-day tests in most hospitals that have their own clinical laboratories. This means that the first step for outpatients in the morning is to visit the blood drawing room.

The test results must be available by the time they see their doctors, typically within approximately one hour.

Table 3. Likely reasons limiting the routine use of LC/MS in the clinical chemistry field in Japan

- The advantages of MS-based assays over conventional methods are not well recognized among medical professionals.
- The vast majority (>99%) of laboratory tests for outpatients are performed as same-day tests, demanding high throughput and rapid turnaround time.
- Most LDTs are not reimbursable in Japan
- LC/MS/MS is not fully automated; its workflow is labor-intensive and not user-friendly
- Inter-laboratory variation of LC/MS/MS is not well clarified.

Achieving this rapid turnaround time, no greater than 60 min, is mandatory, which is currently a challenging task for most LC/MS-based procedures.

4.3. Issues of reimbursement (coverage by health insurance)

MS-based laboratory tests are officially reimbursable only under the following circumstances at the moment in Japan.

1) Identification of microorganisms by MALDI TOF-MS
2) Confirmatory tests for inborn errors of metabolism
3) Toxicology and TDM under certain limited conditions

In the field of clinical chemistry, certain test items such as homocysteine and Vitamin B1 and B2 are reimbursable when determined by laboratory-developed LC/MS based methods. This is because authorized in vitro diagnostics are currently not available for these items. In this context, it is noteworthy that urinary steroid profile analysis is conducted on a routine basis under health insurance coverage at Keio University Hospital[19].

For other test items where certified In Vitro Diagnostics (IVDs) are available, laboratory tests conducted using laboratory-developed MS-based methods are not currently covered by the health insurance system in Japan. This situation causes various problems in clinical practice. For example, IVDs for androstendione and corticosterone used to be commercially available in Japan, but they are now off the market. In clinical situations where measurements of these steroids are required, one must obtain the data using laboratory-developed LC/MS/MS methods without health insurance coverage.

These situations contrast with those in Western countries. Notable differences exist in the regulation of medical devices between Japan and the United States. In the United States, laboratory-developed tests (LDTs) have been reimbursable when developed and conducted by a clinical laboratory authorized by the Clinical Laboratory Improvement Amendments (CLIA).

Recently, however, the Food and Drug Administration (FDA) has proposed to amend its regulations to explicitly state that in vitro diagnostic products (IVDs) are devices under the Federal Food, Drug, and Cosmetic Act, including when the manufacturer of the IVD is a laboratory. The majority of clinical laboratories in the U.S. oppose this FDA regulatory framework for LDTs[20].

LDTs have not yet been clearly defined in Japan. They are not eligible for coverage under public health insurance unless the equipment used is officially approved as an in vitro diagnostic medical device.

In the field of clinical genetics, laboratory-developed genetic testing for only a limited number of monogenic and intractable diseases is exceptionally reimbursable. However, genetic tests essential for the accurate diagnosis of most other genetic diseases are not reimbursable. I believe it is now time for the fields of laboratory medicine and clinical genetics to work together to address the reimbursement issues of LDTs. A national-level discussion is necessary regarding the evaluation and handling of LDTs in Japan. It is expected that the Committee for Promotion and Standardization of Mass Spectrometry-Based Clinical Assays within the Japanese Society for Biomedical Mass Spectrometry (JSBMS) can play significant roles in this matter.

4.4. Limited full automation of the LC/MS/MS system

The workflow of LC/MS/MS remains labor-intensive and primarily manual, requiring highly trained personnel. Consequently, this technology has been limited to academic institutions and highly specialized laboratories. It is clear that achieving full automation of the LC/MS/MS system, preferably with a push-button design and bidirectional access to the Laboratory Information System, is needed[21].

The Shimadzu Corporation has achieved significant success by developing fully automated LC-MS/MS systems. These automated systems have been validated in various areas, including therapeutic drug monitoring (TDM)[22], steroid measurements[23], and 25-hydroxy vitamin D assays[24]. Thermo Fisher has also developed a fully automated LC-MS/MS instrument[25], although it is not currently on the market. More recently, Roche Diagnostics is planning to launch an automated mass spectrometry clinical analyzer[26]. Although the details, including actual data, have not been revealed, their announcement has attracted considerable attention.

In addition to the full automation of LC-MS/MS systems, the wide availability of IVD reagents, including calibrators and preanalytical reagents, will significantly enhance the adoption of LC/MS/MS in the field of clinical chemistry.

4.5. Inter-laboratory variation of LC/MS/MS is not yet fully clarified

Standardization and harmonization of LC/MS/MS-based methods is another crucial step toward wider acceptance of

LC/MS/MS in routine clinical chemistry in Japan.

Due to the nature of MS technology, there has been an assumption that assay results obtained from different MS equipment can be easily harmonized. However, this assumption is incorrect. It is well documented that substantial variability exists between laboratories and instruments[27].

This variability between laboratories and instruments can be attributed to various factors, including: 1) Differences in sample pre-treatment procedures, 2) Differences in LC/MS methods, 3) Diverse types of internal standards and calibrators and 4) Limited full automation of the entire process, requiring substantial direct human intervention

The results of an interlaboratory comparison of 25(OH)D assays by the Vitamin D Standardization Program (VDSP) indicated that only 53% of the LC/MS/MS assays met the VDSP criteria of a mean % bias＜|±5%|. It was shown that the lack of separation between 3-epi-25(OH)D_3 and 25(OH)D_3 significantly impacted these unsatisfactory assay performances[27]. We believe that appropriate LC-separation of various vitamin D metabolites is crucial in the measurement of 25(OH)D_3 by LC/MS/MS. This is particularly important for 25(OH)D_3 measurement in infant and pediatric specimens, which contain significant amounts of the 3-epi form.

The use of a common standard would improve agreement among laboratories using LC/MS/MS methods. To give a representative example, in another international Quality Assessment Scheme (DEQAS) for 25(OH)D, it was demonstrated that the use of a commercially available common standard (Chromosystems Inc, Germany) instead of an in-house standard significantly reduced the mean interlaboratory imprecision (coefficient of variation) from 16.7% (in-house standard) to 8.5% (common standard)[28].

The guideline CLSI C62 (Liquid Chromatography-Mass Spectrometry Methods)[29] is useful, as it covers not only the preparation of calibrators but also method validation specific to mass spectrometry. Calibrators for a limited number of analytes, including vitamin D metabolites, are commercially available from companies such as Chromsystems Instruments and Chemicals GmbH (Munich, Germany). The traceability chains of their calibrators are available on the Chromsystems website[30] Human Serum Standard Reference Material for quality control samples can be obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

In the near future, it is expected that fully automated LC/MS/MS instruments will be released by multiple industries. Each industry has been developing their own unique pretreatment methods and LC/MS/MS systems, and it is likely that they will consider building fully automated systems as an extension of these developments. While automation will significantly contribute to the widespread adoption of LC/MS, we are concerned that automation alone cannot resolve the issue of differences between instruments. It will be necessary to continue conducting surveys using common calibrators verified according to reference measurement procedures to ensure harmonized results[31].

The Committee for Promotion and Standardization of Mass Spectrometry-Based Clinical Assays of the JSBMS has initiated a survey of inter-equipment differences in collaboration with several MS vendors dealing with LC-MS/MS in Japan. As a first step, inter-equipment differences in 25(OH)D_3 and 25(OH)D_2 levels have been assessed (manuscript in preparation).

5. Conclusion

Despite the numerous advantages of LC/MS/MS over immunoassays, its use in clinical chemistry laboratories in Japan remains very limited. To accelerate widespread adoption, it is crucial to achieve full automation of LC/MS/MS systems (preferably with a push-button design) and ensure bidirectional access to the Hospital Information System. Additionally, addressing the reimbursement issues for LDT-based testing is essential. Furthermore, the standardization and harmonization of LC/MS/MS-based methods is another crucial step toward wider acceptance of LC/MS/MS in routine clinical chemistry in Japan.

Conflict of Interest

Fumio Nomura has the following financial relationship to disclose.

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