

Piogenic liver abscess-A challenge for cooperation between family medicine and clinics

Zavidić, Tina; Dejhalla, Ema; Zahirović, David; Lovrinić, Đino

Source / Izvornik: **Journal of Clinical and Experimental Investigations, 2022, 13**

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

<https://doi.org/10.29333/jcei/12190>

Permanent link / Trajna poveznica: <https://urn.nsk.hr/urn:nbn:hr:184:645474>

Rights / Prava: [Attribution 4.0 International](#)/[Imenovanje 4.0 međunarodna](#)

Download date / Datum preuzimanja: **2024-12-02**



Repository / Repozitorij:

[Repository of the University of Rijeka, Faculty of Medicine - FMRI Repository](#)



Review

Mass Spectrometry-Based Techniques Towards OMICS 2.0

Ivan Šoša ^{*}

1, ^{*} Department of Anatomy, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; ivan.sosa@uniri.hr

Abstract The literature was scoped to help place mass spectrometric (MS) platforms in the spotlight of the life sciences, as a tool of OMICS and OMICS 2.0 technologies. These methods were discussed, and their feasibility was highlighted in many reviews that have been periodically published, in addition to the most recently developed term OMICS 2.0. This author's primary interest was the forensic application of MS, but since these interests got broadened, terms such as "biosensors" were also included. So, means of discovery of efficient biomarkers and clinical therapeutic targets were also reviewed. However, within the framework of this review, physical chemistry, bioinformatics, or specific software solutions were not specifically scoped. Aside from simply reviewing the research status and the future perspectives, this article tackles terms such as Society 5.0 and OMICS 2.0. Since the topic of generating permanent collections of digital content for retrospective analysis was reviewed, what requires loads of data – cloud storage is discussed in the context of MS. In this manner, the review has strengthened an in-depth understanding of the applications of MS. These large quantities of data are easier to operate with if generated by MS, rather than by any other commercially available assay.

Keywords: cost; mass spectrometry; OMICS; OMICS 2.0; Society 5.0; sustainability

1. Introduction

One of the universally used - powerful and flexible tools of any laboratory is actually a hybrid of gas chromatography (GC) and mass spectrometry (MS). MS is one of the main analytical technologies on which the "-omics" approaches are based [1,2]. emerging concept of OMICS 2.0 pushes it even further into the foreground [3,4].

Essentially, we use this exquisite combination to separate mixtures of complex composition, count analytes, and identify unknown compounds represented by peaks on spectrogram [5-7]. Both techniques are highly precise and swift, and they both have proven to be exceptionally operative and informative when combined. This appliance is used for analyzing chemical mixtures in drug screening and addiction recovery monitoring, forensic, environmental, and trace analysis and is a technique with supreme sensitivity and selectivity [6,8]. The rationale for this "hybridization" is that it is not possible to make an accurate identification of a particular molecule by a GC or MS alone. However, when these two analytical methods are combined, they allow efficient substance identification [9,10].

2. Forensic Alcohol Determination

Toxicology assessment is performed by the fields golden standard - headspace gas chromatography with flame ionization detection (HS-GC-FID) [11-13]. This method is merely a variation of apparatus drafted in figure 1.

It provides key information as to the type of substance present in an individual and of the amount of those substances [14]. Basically, a person's blood alcohol concentration is a function of the total amount of alcohol in the person's system divided by total body water [15,16]. Though, total body water is the preferred method to use in forensic blood-

alcohol calculations rather than ethanol's volume of distribution. This is so because, alcohol has an affinity for water, so the more water there is in which to distribute the alcohol, the lower the blood alcohol concentration will be.

3. Standard GC-MS

Separation science and analytical technique are combined, and blood is the preferred specimen. Since the GC is a separation technique here, certain outputs of the separation are inputs in the analytical part, and that input has its features. For instance, the interval between the injection of a sample into the glass coil and the detection of substances in that sample is called retention time (RT). It's the unique and substance-specific time required for the solute to pass through a chromatographic column [17]. Most often, separation and analysis devices are merged (hyphenated) into a unique instrument via a heated transfer line. For that reason, we call these techniques – “hyphenated” analytical techniques. The GC utilizes a capillary column (figure 1). The separation abilities of that capillary column depend on the column's dimensions as well as the properties of the analyte. Individual feature of components constituting analyte will stimulate the separation of the molecules as the sample/ analyte travels the length of the column.

Each molecule is further broken by MS into ionized fragments and fragments are identified using their mass-to-charge ratio [10]. This can be used to determine the molecular weight and elemental composition, thus chemical structures of molecules. Each ion has its particular mass-to-charge ratio.

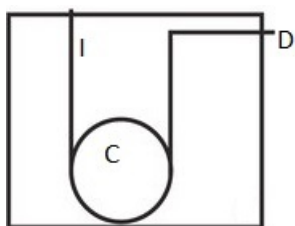


Figure 1 Schematic of standard GC-MS; (I) – input/ injection; (C) – capillary column; (D) – detection device

The usefulness of hyphenated instrument is evident in many separation processes as they are common tools in industry and research.

4. Semiquantitative Widmark equation

Still, the first and the most popular calculation method is Widmark equation [18] which is based on oxidation-reduction reaction and amount of ethanol is calculated indirectly. This equation is the most common form of the equation to estimate the blood alcohol concentration. It utilizes a known amount of bichromate solution (potassium bichromate and acidum sulfuricum), followed by titration of superfluous bichromate with hyposulphate solution.

It considers:

- the hypothetical initial blood alcohol concentration (before any metabolism has occurred).
- amount of pure ethanol consumed.
- fraction of blood volume that is water.
- total body water of an individual [15,19,20].

The simplified version of the Widmark equation is: blood-alcohol concentration = [Alcohol consumed in grams / (Body weight in grams x r)] x 100.

In this formula, "r" is the gender constant, and equals 0.55 for females and 0.68 for males [21].

5. „OMICS“

The word „OMICS“ refers to a field of study in biological sciences that ends with -OMICS, such as genomics, transcriptomics, proteomics, or metabolomics [22]. It is a vernacular expression - suffix -OMICS is used frequently to describe something big and refers to a field of study in life sciences that focuses on large-scale data/information - such as proteomics, genomics, metabolomics, etc. [23]. Proteomics and genomics were the first „OMICS“ formed to date, and now there are lots of closely related scientific disciplines. Some of these disciplines gave birth to new scientific fields and covered narrower arrays of a specific scientific field [24].

Approaches based on MS and steered to answering the problems of petroleomics, foodomics, and economics; or those problems related to inorganic sciences - are merely mentioned. The most compelling consideration is paid to „OMICS“ related to the life sciences as those represent the majority of today's „OMICS [2]. Notwithstanding this explanation, there are „OMICS“ outside of the life sciences circle. On the other hand, working with copious data, whatsoever, is not complete without statistical, and mathematical processing of databases. Generally speaking, MS laid a foundation for the set of „OMICS“ disciplines, and many of them took shape as sciences after some new advances in the field of MS [25].

Additional advances of a scientific culture and responsible innovation is called OMICS 2.0. It is driven by systems-oriented thinking, meta-analysis, and independent social science analyses that help bridge the current gaps between omics technologies and society [3].

6. Possibilities

GC-MS can be used for an assortment of applications comprising the detection of potentially toxic chemicals in foods the quantitation of organic contaminants in water, the determination of pesticide residues to the characterization of ingredients [26]. As previously said on many occasions, distinguishing compounds of a mixture to separate them is performed by injecting a gaseous sample (analyte) into a carrier gas (usually an inert or an unreactive gas). With this procedure, a mobile phase is produced. this is a gaseous mixture driven through a capillary column - stationary phase [10].

It was not until 1977 that GC-MS was admitted to the court as evidence [27], and nowadays GC-MS can be used to study liquid, gaseous or solid samples. The analysis begins with the GC, where the sample is effectively vaporized into the mobile phase and separated into its various components using a capillary column coated with a stationary (liquid or solid) phase. As elements of our analyte are separated, each element drops down the column at a different time based on its properties. Once the components leave the GC column, they are ionized and fragmented by the MS using electron or chemical ionization sources. Ionized molecules and fragments are then accelerated through the instrument's mass analyzer. It is here that ions are separated based on their different m/z ratios. In the context of metabolome/ metabolomics, MS-based laboratory techniques should be regarded only as a tool for researchers to dive deeper into the metabolome and explore all metabolites.

Dealing with substances that will be detected at very low levels, or with will be very little information about their toxicity, would result in controversies over whether the concentrations found pose a significant risk to the public health or the environment, he added Drug testing, as an example, can be carried out for multiple reasons: pathology, healthcare, and the anti-doping of both humans and animals [28]. Their concentration in biological fluids is usually very low, so their detection requires the GC-MS, additionally, the molecules in merit are often very small (<650 Daltons) [8,29,30].

however, compound identification and reliable quantification are greatly complicated owing to the chemical complexity and dynamic range of the metabolome [31]. GC-MS data acquisition can be performed to enable the detection and quantification of many thousands of metabolite features as part of MS-based metabolomics.

7. MS-based quantitative strategies and analysis of proteome, genome, and transcriptome

Cells are continuously operational biochemical laboratories, and in their versatility, biological systems are incredibly complex. The result is an almost inexhaustible object of research [2]. The term “genome” was something that changed throughout history, so instead of the totality of genes in all chromosomes (what was its original meaning), it now designates the complete set of DNA in organisms, including all of its genes [2]. The elementary monomeric units of DNA and RNA macromolecules are nucleotides, consisting of nitrogenous bases, monosaccharides, and phosphoric acid residues. Molecules of purine (adenine, guanine) and pyrimidine (thymine, cytosine) structures act as nitrogenous bases for DNA. The same bases are included in the nucleotides of RNA molecules, except that thymine in them is replaced by uracil. In DNA and RNA, these nitrogenous bases bind to monosaccharides deoxyribose and ribose, respectively, forming nucleosides. In nucleotides, the latter is phosphorylated at the primary OH group of a monosaccharide, and this phosphate [32].

Even though qRT-PCR is considered the gold standard in transcriptome techniques due to its accuracy and sensitivity, RNA-seq turns out to be equivalent[16,17]. Transcriptomics can either refer to exploratory analysis of the entire transcriptome, primarily using RNA sequencing (RNA-seq) [33], or to targeted analysis of known RNAs using techniques such as gene expression panels (GEPs).

Of all MS-based quantitative strategies, in the analysis of nucleic acids most often used is matrix-assisted laser desorption/ionization– MS (MALDI-MS), recently improved with the time-of-flight (ToF) feature [34]. This appliance should nevertheless recoil due to high initial costs, especially since it does not require trained laboratory personnel [35]. Interestingly, RNAs are more stable than DNA, so the latter are preliminarily converted into RNA by in vitro transcription.

The scope of delivery of RNAs formed in one or a series of cells of an organism is called a "transcriptome" [36,37]. It consists of ribosomal (rRNA), transport (tRNA), and messenger (mRNA) RNA [38,39]. Out of those three forms of RNA, mRNA serves as a transmitter of genetic information [40,41], After translation, these RNAs are rapidly degraded. Though, the complete transcriptome is a subject of interest in transcriptomics. [42]. During translation, tRNAs take part as an intermediate between nucleic acids and proteins: they attach activated amino acid residues and transfer them to the site of synthesis of protein polypeptide chains rRNAs do not participate in the process of information transfer and constitute the bulk of the ribosomes. The sequence of nucleotides in mRNA molecules contains all the information about the amino acid sequence in a protein chain.

All types of RNA are synthesized on a DNA template, and the sequence of ribonucleotides in them is complementary to the sequence of deoxyribonucleotides in DNA. Molecules of purine (adenine, guanine) and pyrimidine (thymine, cytosine) structures act as nitrogenous bases for DNA. The elementary monomeric units of DNA and RNA macromolecules are nucleotides, consisting of nitrogenous bases, monosaccharides, and phosphoric acid residues. Though, the same bases are included in the nucleotides of RNA. Except that thymine is replaced by uracil. In DNA and RNA, these nitrogenous bases bind to monosaccharides deoxyribose and ribose, respectively, forming nucleosides. Monosaccharides deoxyribose or ribose are phosphorylated in nucleotides, and this phosphate group phosphorylates the OH group of a monosaccharide of another nucleotide, because of which macromolecules of nucleic acids are formed.

Quantitative strategies relying on MS and MS within genomics/transcriptomics are only logical. MS led to the concept of "OMICS". And nowadays sequencing and MS are basic experimental tools in investigating the omics of a given biological system. Since a substantial amount of information is obtainable from sequencing-based methods, MS-based techniques can be used to investigate proteome, metabolome, and interactomes that do not involve DNA/RNA [43].

An enormous number of processes simultaneously occur in all of our cells. Accordingly, the proteome is more of a challenge than the human genome [44]. Not only its scale is estimated at over 1 million proteins but the increase in proteomic diversity is further increased by protein post-translational modifications (PTMs) [45]. Precisely the analysis of PTMs could provide a comprehensive vision of molecular mechanisms for various diseases [46]. Though, these modifications of proteins following protein biosynthesis are generally enzymatic. It refers to changes in the polypeptide chain as a result of adding distinct chemical parts to amino acid residues.

To be blunt, PTMs are the foundation of complicated cellular processes, such as cell division, growth, differentiation, signaling, and regulation, the same as various processes included in the maintenance of protein structure and integrity [47].

PTMs also regulate the metabolism and defense processes, cellular recognition, and morphology alternation [48]. Consequently, analysis of PTMs is important for the study of cell biology and disease diagnostics and prevention. The worldwide studies of different PTMs and the proportion change of different proteins could provide new insights into the clinical approach [24]. To highlight the relevance of MS in the analysis of PTM, statistics of each PTM experimentally and putatively detected have been compiled using proteome-wide information from the Swiss-Prot database [49].

Many physiological functions can be overviewed using MS-based quantitative strategies developed to quantify glycoprotein expression levels on a large scale [50]. Proteomics based on MS is made feasible by the availability of gene and genome sequence databases and technical and conceptual advancements in many areas [51-54]. Typically, MS can cover a range of functions [55]. Time-of-flight (TOF), quadrupole (Q), Fourier transforms ion cyclotron resonance (FT-ICR), and ion trap (IT) that are used generally in labs, are usually combined in proteomics in one MS (triple quadrupole (QqQ), Q-IT, Q-TOF, TOF-TOF, IT-FTMS, etc.) [56].

8. MS-based quantitative strategies, food, and environment

Food science and nutrition interact with disciplines such as pharmacology, medicine, and biotechnology. Conversely, the discipline that we call environmental health, specifically environmental toxicology provides critical information and knowledge for regulatory agencies, decision-makers, and others. To make that

possible, scientists may take advantage of MS. Today's appliances aim to identify and quantify complex protein (peptides) mixtures in a single experiment [57,58].

in which modern analytical chemistry must provide accurate, precise, and robust methods to determine any harmful compounds or organisms that might be present in food at very low concentrations. Detection of exogenous contaminants in food, food safety, quality, and traceability with MS-based "OMICS" is always a complex area.

Foodomics is a portmanteau coined in 2009 as "a discipline that studies the Food and Nutrition domains through the application and integration of advanced -OMICS technologies to improve consumer's well-being, health, and knowledge". FoodOMICS requires a combination of food chemistry, biological sciences, and data analysis [59].

A framework of knowledgeability required for the average medical student in the field falls greatly beyond the topic of food cOMICS, so this review shall focus specifically on MS and its application.

In the framework of environmental health, MS is mainly the environmental toxicology tool used to put programs and policies in place to limit our exposure to these substances, thereby preventing or reducing the likelihood that a disease or other negative health outcome would occur [60]. MS has its inevitable position in wastewater-based epidemiology for the determination of small and large molecules as biomarkers of exposure [61,62]. A systematic review of MS and OMICS

To conduct this review, Web of Science Core Collection, PubMed, and Jisc Library databases were searched for MS and OMICS, sustainability, and cost.

From the published articles, only those that appeared after 2015 were selected. The initial search (with "AND" as an operator) yielded 51 results from the Web of Science Core Collection. 36 results were found in PubMed, and 5 document results were found in the Jisc Library databases.

From the total number of 92 articles, we excluded 20 duplicates and 6 unrelated articles. The remaining 66 articles were all written in English, no article included a single case report. A total of 66 articles were used for this systematic review and the PRISMA flow diagram can be seen in figure 2.

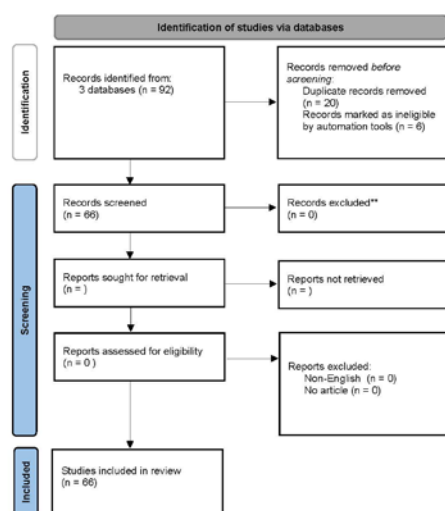


Figure 2 PRISMA diagram of the systematic review of the published articles, only those that appeared after 2015 were selected with "and" as an operator

9. Immunoassay or MS- Affordability

Immunoassays use specific antibodies, while MS exploits measuring the mass-to-charge ratio of ionized molecules to quantify an analyte. Many clinical analytes can be measured using either immunoassay or mass spectrometric methods and choosing the method to use can be a demanding decision dependent on a variety of factors. Most shortened, immunoassays are traditionally used for larger molecules (eg, proteins) while MS is more commonly used to detect small molecules (eg, drugs) [63].

Some laboratories employ both techniques, but generally choice depends on clinical need. Immunoassays are generally reliable and accurate; they are more automated and decrease the sample preparation required. Considering the findings of Liu et al. and the effects of deep learning on the computational time of the algorithm, the use of deep learning in the MS should be encouraged [64]. For the most part, MS-based methods have lower limits of quantification than immunoassays and are therefore useful for measuring very low analyte concentrations [65]. Conversely, due to its potential to provide greater analytical sensitivity and specificity MS is increasingly used, especially at low levels [54].

Interfering substances and sample impurities alter the measurable concentration of an analyte, can be relatively common, and vary widely between patients and assay platforms. Although the matrix may affect both immunological and mass spectrometric methods, the effects tend to be more prevalent in immunoassays because sample extraction or separation methods are less common.

Immunoassays are generally commercially developed, while mass spectrometric methods are often laboratory-developed and can therefore be substantially different from one another [66-68]. Both methods suffer from this lack of standardization. Considering the variety of assay platforms, the lack of standardization and discrepant results between laboratories without actual reference intervals are understandable. Competing manufacturers independently develop and offer a variety of assay platforms, reagents, antibodies, and standards, which all contribute to different results and reference intervals. Conversely, mass spectrometric methods are often laboratory-developed and can therefore be substantially different from one another.

10. Feasibility

Regardless of its importance in substance identification [9], when deciding between immunoassay and MS for other analyses, the best option will depend on the needs, goals, and patient population.

The chief disadvantage of MS methods is that they are not easily integrated into core laboratory automation. Additionally, they require personnel (although, the “new” MALDI-ToF does not) with specialized training, and, due to batch processing, tend to have longer turnaround times (which has been successfully reduced). In contrast, immunoassays tend to be more easily automated and require less training but are more susceptible to interference and can require higher-priced reagents, hence costing more to run [35,64,69,70].

On the other hand, the cost of running LC-MS practically does not exist, however financial considerations for purchasing an MS are substantial. After explaining the financial components that must be considered when purchasing an MS system, it is unavoidable to calculate the return on investment [71,72]. Insignificant operating expenses in comparison to intimidating initial costs could ultimately be used as an excuse, together with an argument that currently there is no alternative in the market [73,74].

To evaluate the cost-effectiveness and return on investment, when deciding on investing in a new MS instrument, the price of an MS instrument, as well as all other (recurring) costs, should be considered (i.e., service contract, reagents, consumables, etc.). that are necessary to keep a workflow current with access to technology advancements, as well as service, training, and support. Before making such a decision, it is

necessary to insure against equipment obsolescence [75,76]. A systematic review of MS and sustainability/MS and cost

To finalize this review with data on sustainability, Web of Science Core Collection, PubMed, and Jisc Library databases were searched for MS and, sustainability; with “and” as a logical operator. The search returned 3 items. Unfortunately, they were outdated, so excluded from the literature review.

Simply put, the fact that the literature search yielded no results, or no studies make its publication harder. However, its value is to invite researchers to contribute to the field.

Sustainable MS-based quantitative strategies

MS is a key analytical technique in receiving the required molecular information from minute amounts of samples present in the gas, solid, or liquid phase. This is crucial for many applications in life science and environmental science-oriented „OMICS”. Conclusively, progress in all these fields would contribute to the long-term vision originating from the project’s objectives, specifically molecular isomer differentiation or weighing the chemical bonds in a molecule directly by MS. All these approaches could leave a deep impact on physical, (bio)chemical, and life sciences. The envisioned scientific achievements in health and sustainability applications may translate into improved drug discovery, early diagnosis, and prognosis for preventive and personalized medicine. The planet and humanity should further benefit from an increase in the sustainability of energy production and processing.

Sounded alarms of various governmental or nongovernmental organizations and independent researchers alerted us of rapid and global progress that could seal this generation’s and generations to come fate. Namely, we could all face a future steeped in inequity and climate crises. As MS is practically zero-waste technology we could all pull our weight without compromising the ability of future generations to meet their own needs. Without actually knowing what sustainability looks like in practice.

Therefore, perhaps it is sound to expect that machine learning algorithms and deep learning could further improve the sustainability of MS since it improves library retrieval accuracy, the accuracy of spectral library retrieval, and reduces the computational time of the algorithm by more than 2 hours in the study of Liu et al. [64].

Everyone wants it, but all may have different ideas in their heads of what that means. However, it will need a whole collection of ambitious strategies to achieve meaningful change. So, it is not a matter of asking how exactly to get there, but to start walking.

Even if favoriting MS-based quantitative strategies is a small step for overall sustainability, it is the start of a long journey toward a sustainable future, and it may turn out to be an unparalleled notch-up for some future pandemics [77,78]. At the moment, considerable efforts are invested in the development of sustainable nanosystems as front-end technology for MS [79].

Likewise, in the framework of the Community Research and Development Information Service (CORDIS) of the European Commission, there is a project funded by the EU's framework programs for research and innovation of the rapid screening and identification of illegal drugs by IR absorption spectroscopy and gas chromatography as well as an initiative to redefine MS into a breakthrough platform for real-time noninvasive breath analysis with single ion detection of intact viruses and bacteria and post-analysis molecular characterization, so Ms has a future as a sustainable method of the tomorrow [80-82].

11. A systematic review of MS and sustainability/ mass spectrometry and sustainability

To finalize this review with data on sustainability, Web of Science Core Collection, PubMed, and Jisc Library databases were searched for: MS and, sustainability; with “AND” as a logical operator In the next query, “sustainability” was replaced with “sustainability”.

No results at all were referring to this query, so like in the previous case, the literature search yielded no results, or no studies were included in our scope.

12. MS and OMICS 2.0 in the Society 5.0

The extremely new concept of Society 5.0 is like a guide to social development and can have a profound impact on all points of society, as it emphasizes the potential of the individual-technology relationship [83]. We gave such a catchy name to the “super-smart” society that does not bother with lame questions like “ELISA or MS?”. Indeed, just like Industry 4.0, which is the digital transformation of manufacturing, Society 5.0 aims to tackle several other challenges by going far beyond just the digitalization of the economy [84-86]. Digitalization in the present context extends to all levels of society and ITS (digital) transformation. The omnipresent diffusion of new technologies has recently led to a dramatic adjustment of the techniques and technologies in almost all countries [87]. Internet, robots, self-driving cars, and artificial intelligence are all “new” risks [88-90]. Therefore, it has a number of pretty comprehensive consequences even for this “ELISA or MS” dilemma. For instance, the emerging “digital ELISA” (single-molecule detection platform) can be combined with the concept of multiplexing. In this way, extremely low serum concentrations of proteins detectable by “digital ELISA” are rendered even more convenient by adding the option of employing multiple analytes, using several types of nanomaterial-based biosensors [91,92]. In the other hand, MS has become a standard for the analysis of different substances in the clinical laboratory [93].

Aside its unique strength in performing analysis, MS has a remarkable ability of generating permanent collections of digital content for retrospective analysis [94]. Substances such as antibiotics, immune-suppressive drugs, drug metabolites in former addicts, or therapeutic antibodies used for the treatment of different diseases can all be tracked by the MS. High-resolution, mass-accurate data can demand infrastructure capable of managing 1 GB/h of data. Such enormous quantities of data are generated not only by life science investigators but, increasingly, by those working in industries that depend on high-volume processes like characterizing the presence of metabolites and their biotransformations. In Society 5.0 application of OMICS technologies is tackled in diverse and complementary global settings. We call this concept - OMICS 2.0 [3,25]. This leap forward furthers the improvement of the quality of life of all people in a sustainability world [95]

Talking specifically of MS, resulting RTs and m/z can be summed, plotted and stored as a series of data acquired by modern digital instruments. Collections of data yielded from such a material can be made available in cloud computing model that enables storing data and files on the internet through a cloud computing provider [96,97]. With data obtained by MS-based quantitative strategies, it is feasible to identify those compounds. However, recent works, such as that of Meissner et al. herald its use in proteomics [54]. Samples are generally dissolved or diluted in a solvent and then injected into the heated inlet port. However, some other methods of sample preparation may also be involved [98]. The liquid sample is vaporized in the hot inlet and converted to gas [99]. The separated compounds then leave the column and enter a detector. The time taken to travel through the column and reach the detector is called retention time [100]

13. Machine learning and artificial intelligence (AI) approaches

Machine learning and artificial intelligence (AI) approaches are indispensable components of this concept and have revolutionized multiple disciplines, including toxicology [101,102]. AI-influenced artificial neural

networks (ANN) can be employed in forensic science analysis for various purposes. Basically, it is the use of multiple weighted inputs that are summed to produce an output that is then analyzed [103]. AI-augmented toxicology lab offers the most elegant method of dodging space limitations since the need to store several thousands of skeets in large physical archives practically waved [104,105]. Evidently, AI has the potential to outperform most forensic medicine specialists skilled in toxicology in the setting of a toxicological laboratory [106]. Thus, it may soon automate and standardize. Though plots used in AI datasets are visually different from the original, however, proper demands for data storage make CNN optimal in this regard [12,107-109]. Deep learning AI models are currently being used in analytical procedures as an assessment to help with efficiency, consistency, and decision-making. Unfortunately, they still keep the forensic specialist skilled in toxicology at the center of the assessment. By all accounts, it will remain so, at least shortly [110,111].

Present state-of-the-art toxicology is far away from some anecdotal attainments [112-114]. Learning from the input data mimics the functioning of neurons and their communications to convey complex behavior. This progress follows the progress in the preparation and processing of input material, accessibility of large datasets goes hand-by-hand with the expansion in algorithms' structure. On the other hand, progress in computing programming ignited diligence for learning on the AI-constructed machines built for high-dimensional output of data [115]. A model for the near future comprises the forensic medicine specialist skilled in toxicology enhanced by a real-time artificial intelligence system's second review [116,117].

We propose a novel strategy of deep learning in the toxicological laboratory. Machines trained and validated by toxicology, from subjects that covered diverse and representative clinical cases, as seem usually in everyday practice [118]. This would yield an AI system that can handle a large number of toxicological reports without potential disturbances commonly experienced by professionals in the field (space or time limitations, for instance) [119,120]. It will drastically alleviate the heavy clinical burden of daily work. This would also be a generalizable tool for other professions with similar background knowledge.

14. Conclusions

Currently, the concept of systems biology prevails on the big scientific scene and is the talk of the town in biology. Leading methods of such scientific approach include interrelated „OMICS” sciences: transcriptomics, genomics, proteomics, and metabolomics. Perhaps we should all have great hopes for the smooth transition of MS to deep learning, environment because that environment has proven to eliminate timewasters, rendering MS an out-of-favor method. Results of MS yielded in the scope of these „OMICS” sciences generally generate a large number of permanent collections of digital content for subsequent analysis, why this method is increasingly attractive in the digitalized society...

For time being no single method is generally preferred. When deciding between immunoassay and MS-based techniques, the best option will depend on the needs, goals, and patient population. Both methods are subject to impurities and contaminations, but the effects tend to be the most prevalent in immunoassays. Maybe, it is time to introduce MS-based strategies to an approach to gathering information from several “omes (“multi-OMICS”).

Funding: The APC was funded by the University of Rijeka, Faculty of Medicine

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable

Acknowledgments: This author expresses the kindest thank you to the University of Rijeka, Faculty of Medicine.

Conflicts of Interest: None

References

1. Di Girolamo, F.; Lante, I.; Muraca, M.; Putignani, L. The Role of Mass Spectrometry in the "Omics" Era. *Curr Org Chem* **2013**, *17*, 2891-2905, doi:10.2174/1385272817888131118162725.
2. Zaikin, V.G.; Borisov, R.S. Mass Spectrometry as a Crucial Analytical Basis for Omics Sciences. *Journal of Analytical Chemistry* **2021**, *76*, 1567-1587, doi:10.1134/S1061934821140094.
3. Ozdemir, V. OMICS 2.0: An Accelerator for Global Science, Systems Medicine and Responsible Innovation. *OMICS* **2015**, *19*, 579-580, doi:10.1089/omi.2015.0133.
4. Ozdemir, V. OMICS 2.0: a practice turn for 21(st) century science and society. *OMICS* **2013**, *17*, 1-4, doi:10.1089/omi.2012.0111.
5. Pacot, G.M.M.; Lee, L.M.; Chin, S.T.; Marriott, P.J. Introducing Students to Gas Chromatography-Mass Spectrometry Analysis and Determination of Kerosene Components in a Complex Mixture. *Journal of Chemical Education* **2016**, *93*, 742-746, doi:10.1021/acs.jchemed.5b00830.
6. Proadhan, M.A.I.; McClain, C.; Zhang, X. Comprehensive Two-Dimensional Gas Chromatography Mass Spectrometry-Based Metabolomics. *Adv Exp Med Biol* **2021**, *1280*, 57-67, doi:10.1007/978-3-030-51652-9_4.
7. Yost, R.A. Why tandem mass spectrometry for trace analysis: Concepts of tandem analytical techniques. *Rapid Commun Mass Spectrom* **2022**, *36*, e9310, doi:10.1002/rcm.9310.
8. Zeki, O.C.; Eylem, C.C.; Recher, T.; Kir, S.; Nemetlu, E. Integration of GC-MS and LC-MS for untargeted metabolomics profiling. *J Pharm Biomed Anal* **2020**, *190*, 113509, doi:10.1016/j.jpba.2020.113509.
9. Ferreira, A.B.; Castro, A.L.; Tarelho, S.; Domingues, P.; Franco, J.M. GC-MS - Still standing for clinical and forensic analysis: validation of a multidrug method to detect and quantify illicit drugs. *Australian Journal of Forensic Sciences* **2021**, 1-22, doi:10.1080/00450618.2021.1964598.
10. Harvey, D. *Modern analytical chemistry*; McGraw-Hill New York: 2000; Volume 1.
11. Chun, H.J.; Poklis, J.L.; Poklis, A.; Wolf, C.E. Development and Validation of a Method for Alcohol Analysis in Brain Tissue by Headspace Gas Chromatography with Flame Ionization Detector. *J Anal Toxicol* **2016**, *40*, 653-658, doi:10.1093/jat/bkw075.
12. Jones, A.W. Alcohol, its analysis in blood and breath for forensic purposes, impairment effects, and acute toxicity. *Wiley Interdisciplinary Reviews: Forensic Science* **2019**, *1*, e1353.
13. Mihretu, L.D.; Gebru, A.G.; Mekonnen, K.N.; Asgedom, A.G.; Desta, Y.H. Determination of ethanol in blood using headspace gas chromatography with flameionization detector (HS-GC-FID): Validation of a method. *Cogent Chemistry* **2020**, *6*, 1760187, doi:Artn 1760187
10.1080/23312009.2020.1760187.
14. Fischer, I.; Milton, C.; Wallace, H. Toxicity testing is evolving! *Toxicol Res (Camb)* **2020**, *9*, 67-80, doi:10.1093/toxres/tfaa011.
15. Maskell, P.D.; Korb, A.S. Revised equations allowing the estimation of the uncertainty associated with the Total Body Water version of the Widmark equation. *J Forensic Sci* **2022**, *67*, 358-362, doi:10.1111/1556-4029.14859.
16. Maskell, P.D.; Cooper, G.A.A. The Contribution of Body Mass and Volume of Distribution to the Estimated Uncertainty Associated with the Widmark Equation. *J Forensic Sci* **2020**, *65*, 1676-1684, doi:10.1111/1556-4029.14447.
17. Kapila, S.N.; Boaz, K.; Natarajan, S. The post-analytical phase of histopathology practice: Storage, retention and use of human tissue specimens. *Int J Appl Basic Med Res* **2016**, *6*, 3-7, doi:10.4103/2229-516X.173982.
18. Simpson, G. Medicolegal alcohol determination: Widmark revisited. *Clin Chem* **1988**, *34*, 888-889.

19. Gorgus, E.; Hittinger, M.; Schrenk, D. Estimates of Ethanol Exposure in Children from Food not Labeled as Alcohol-Containing. *J Anal Toxicol* **2016**, *40*, 537-542, doi:10.1093/jat/bkw046.
20. Jones, A.W. Comment on 'Estimates of Non-Alcoholic Food-Derived Ethanol and Methanol in Human'. *J Anal Toxicol* **2022**, *46*, e48-e51, doi:10.1093/jat/bkab112.
21. Brick, J.; Bennett, W. Alcohol Calculations in Emergency and Forensic Medicine. *J Addict Med Ther Sci* **2017**, *3*, 024-029.
22. Vailati-Riboni, M.; Palombo, V.; Loor, J.J. What are omics sciences? In *Periparturient diseases of dairy cows*; Springer: 2017; pp. 1-7.
23. Smejkal, G.B. I'm an -omics, you're an -omics... *Expert Review of Proteomics* **2006**, *3*, 383-385, doi:10.1586/14789450.3.4.383.
24. Janiszewska, D.; Szultka-Mlynska, M.; Pomastowski, P.; Buszewski, B. "Omic" Approaches to Bacteria and Antibiotic Resistance Identification. *Int J Mol Sci* **2022**, *23*, 9601, doi:10.3390/ijms23179601.
25. Wang, J.; Pursell, M.E.; DeVor, A.; Awoyemi, O.; Valentine, S.J.; Li, P. Portable mass spectrometry system: instrumentation, applications, and path to 'omics analysis. *Proteomics* **2022**, *22*, e2200112, doi:10.1002/pmic.202200112.
26. Xu, M.L.; Gao, Y.; Wang, X.; Han, X.X.; Zhao, B. Comprehensive Strategy for Sample Preparation for the Analysis of Food Contaminants and Residues by GC-MS/MS: A Review of Recent Research Trends. *Foods* **2021**, *10*, doi:10.3390/foods10102473.
27. Tummers, A.; Erdmann, R.G. The Eye Versus Chemistry? From Twentieth to Twenty-First Century Connoisseurship. In *Analytical Chemistry for the Study of Paintings and the Detection of Forgeries*; Springer: 2022; pp. 3-45.
28. Sengupta, P.; Chatterjee, B.; Tekade, R.K. Drug toxicity and forensic pharmacokinetics. In *Pharmacokinetics and Toxicokinetic Considerations*; Elsevier: 2022; pp. 425-486.
29. Piechocka, J.; Wiczorek, M.; Glowacki, R. Gas Chromatography-Mass Spectrometry Based Approach for the Determination of Methionine-Related Sulfur-Containing Compounds in Human Saliva. *Int J Mol Sci* **2020**, *21*, doi:10.3390/ijms21239252.
30. Fiehn, O. Metabolomics by Gas Chromatography-Mass Spectrometry: Combined Targeted and Untargeted Profiling. *Curr Protoc Mol Biol* **2016**, *114*, 30 34 31-30 34 32, doi:10.1002/0471142727.mb3004s114.
31. Aseekh, S.; Aharoni, A.; Brotman, Y.; Contrepolis, K.; D'Auria, J.; Ewald, J.; J, C.E.; Fraser, P.D.; Giavalisco, P.; Hall, R.D.; et al. Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices. *Nat Methods* **2021**, *18*, 747-756, doi:10.1038/s41592-021-01197-1.
32. Cofsky, J.C.; Knott, G.J.; Gee, C.L.; Doudna, J.A. Crystal structure of an RNA/DNA strand exchange junction. *PLoS One* **2022**, *17*, e0263547, doi:10.1371/journal.pone.0263547.
33. Rao, A.; Barkley, D.; Franca, G.S.; Yanai, I. Exploring tissue architecture using spatial transcriptomics. *Nature* **2021**, *596*, 211-220, doi:10.1038/s41586-021-03634-9.
34. Li, D.; Yi, J.; Han, G.; Qiao, L. MALDI-TOF Mass Spectrometry in Clinical Analysis and Research. *ACS Measurement Science Au* **2022**.
35. Rychert, J. Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. *Journal of Infectiology and Epidemiology* **2019**, *2*.
36. Fernandez-Pozo, N.; Haas, F.B.; Gould, S.B.; Rensing, S.A. An overview of bioinformatics, genomics, and transcriptomics resources for bryophytes. *J Exp Bot* **2022**, *73*, 4291-4305, doi:10.1093/jxb/erac052.

-
37. Raghavachari, N.; Garcia-Reyero, N. Overview of Gene Expression Analysis: Transcriptomics. *Methods Mol Biol* **2018**, *1783*, 1-6, doi:10.1007/978-1-4939-7834-2_1.
 38. Stark, R.; Grzelak, M.; Hadfield, J. RNA sequencing: the teenage years. *Nat Rev Genet* **2019**, *20*, 631-656, doi:10.1038/s41576-019-0150-2.
 39. Burgess, H.M.; Vink, E.I.; Mohr, I. Minding the message: tactics controlling RNA decay, modification, and translation in virus-infected cells. *Genes Dev* **2022**, *36*, 108-132, doi:10.1101/gad.349276.121.
 40. Yang, J.R. Does mRNA structure contain genetic information for regulating co-translational protein folding? *Zool Res* **2017**, *38*, 36-43, doi:10.13918/j.issn.2095-8137.2017.004.
 41. Mattick, J.; Amaral, P. RNA, the Epicenter of Genetic Information. **2023**.
 42. Komar, A.A. A Code Within a Code: How Codons Fine-Tune Protein Folding in the Cell. *Biochemistry (Mosc)* **2021**, *86*, 976-991, doi:10.1134/S0006297921080083.
 43. Dai, X.; Shen, L. Advances and Trends in Omics Technology Development. *Front Med (Lausanne)* **2022**, *9*, 911861, doi:10.3389/fmed.2022.911861.
 44. Kustatscher, G.; Collins, T.; Gingras, A.C.; Guo, T.; Hermjakob, H.; Ideker, T.; Lilley, K.S.; Lundberg, E.; Marcotte, E.M.; Ralser, M.; et al. Understudied proteins: opportunities and challenges for functional proteomics. *Nat Methods* **2022**, *19*, 774-779, doi:10.1038/s41592-022-01454-x.
 45. Aebersold, R.; Agar, J.N.; Amster, I.J.; Baker, M.S.; Bertozzi, C.R.; Boja, E.S.; Costello, C.E.; Cravatt, B.F.; Fenselau, C.; Garcia, B.A.; et al. How many human proteoforms are there? *Nat Chem Biol* **2018**, *14*, 206-214, doi:10.1038/nchembio.2576.
 46. Li, J.; Zhan, X. Mass spectrometry-based proteomics analyses of post-translational modifications and proteoforms in human pituitary adenomas. *Biochim Biophys Acta Proteins Proteom* **2021**, *1869*, 140584, doi:10.1016/j.bbapap.2020.140584.
 47. Ramazi, S.; Zahiri, J. Posttranslational modifications in proteins: resources, tools and prediction methods. *Database (Oxford)* **2021**, *2021*, doi:10.1093/database/baab012.
 48. Pakhrin, S.C.; Pokharel, S.; Saigo, H.; Kc, D.B. Deep Learning–Based Advances In Protein Posttranslational Modification Site and Protein Cleavage Prediction. In *Computational Methods for Predicting Post-Translational Modification Sites*; Springer: 2022; pp. 285-322.
 49. Malakhova, O.A.; Yan, M.; Malakhov, M.P.; Yuan, Y.; Ritchie, K.J.; Kim, K.I.; Peterson, L.F.; Shuai, K.; Zhang, D.E. Protein ISGylation modulates the JAK-STAT signaling pathway. *Genes Dev* **2003**, *17*, 455-460, doi:10.1101/gad.1056303.
 50. Bagdonaite, I.; Malaker, S.A.; Polasky, D.A.; Riley, N.M.; Schjoldager, K.; Vakhrushev, S.Y.; Halim, A.; Aoki-Kinoshita, K.F.; Nesvizhskii, A.I.; Bertozzi, C.R.; et al. Glycoproteomics. *Nature Reviews Methods Primers* **2022**, *2*, 48, doi:ARTN 48
10.1038/s43586-022-00128-4.
 51. Nesvizhskii, A.I. Proteogenomics: concepts, applications and computational strategies. *Nat Methods* **2014**, *11*, 1114-1125, doi:10.1038/nmeth.3144.
 52. Zhao, Y.; Jensen, O.N. Modification-specific proteomics: strategies for characterization of post-translational modifications using enrichment techniques. *Proteomics* **2009**, *9*, 4632-4641, doi:10.1002/pmic.200900398.
 53. Saeed, F.; Haseeb, M. Need for High-Performance Computing for MS-Based Omics Data Analysis. In *High-Performance Algorithms for Mass Spectrometry-Based Omics*; Springer: 2022; pp. 1-5.
 54. Meissner, F.; Geddes-McAlister, J.; Mann, M.; Bantscheff, M. The emerging role of mass spectrometry-based proteomics in drug discovery. *Nat Rev Drug Discov* **2022**, *21*, 637-654, doi:10.1038/s41573-022-00409-3.

-
55. Li, C.; Chu, S.; Tan, S.; Yin, X.; Jiang, Y.; Dai, X.; Gong, X.; Fang, X.; Tian, D. Towards Higher Sensitivity of Mass Spectrometry: A Perspective From the Mass Analyzers. *Front Chem* **2021**, *9*, 813359, doi:10.3389/fchem.2021.813359.
 56. Lippens, J.L.; Nshanian, M.; Spahr, C.; Egea, P.F.; Loo, J.A.; Campuzano, I.D.G. Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry as a Platform for Characterizing Multimeric Membrane Protein Complexes. *J Am Soc Mass Spectrom* **2018**, *29*, 183-193, doi:10.1007/s13361-017-1799-4.
 57. Herrero, M.; Simo, C.; Garcia-Canas, V.; Ibanez, E.; Cifuentes, A. Foodomics: MS-based strategies in modern food science and nutrition. *Mass Spectrom Rev* **2012**, *31*, 49-69, doi:10.1002/mas.20335.
 58. Guo, H.R.; Hashim, Z.; Su, S.B.; Bundschuh, J. Environmental Toxicology in Addressing Public Health Challenges in East Asia. *Biomed Res Int* **2015**, *2015*, 920518, doi:10.1155/2015/920518.
 59. Cifuentes, A. Food analysis and foodomics. *J Chromatogr A* **2009**, *1216*, 7109, doi:10.1016/j.chroma.2009.09.018.
 60. Stingone, J.A.; Triantafyllou, S.; Larsen, A.; Kitt, J.P.; Shaw, G.M.; Marsillach, J. Interdisciplinary data science to advance environmental health research and improve birth outcomes. *Environ Res* **2021**, *197*, 111019, doi:10.1016/j.envres.2021.111019.
 61. Pico, Y.; Barcelo, D. Mass Spectrometry in Wastewater-Based Epidemiology for the Determination of Small and Large Molecules as Biomarkers of Exposure: Toward a Global View of Environment and Human Health under the COVID-19 Outbreak. *ACS Omega* **2021**, *6*, 30865-30872, doi:10.1021/acsomega.1c04362.
 62. Rahman, A.; Kang, S.; Wang, W.; Garg, A.; Maile-Moskowitz, A.; Vikesland, P.J. Nanobiotechnology enabled approaches for wastewater based epidemiology. *Trends Analyt Chem* **2021**, *143*, 116400, doi:10.1016/j.trac.2021.116400.
 63. Wan, H. An overall comparison of small molecules and large biologics in ADME testing. *Admet and Dmpk* **2016**, *4*, 1-22, doi:10.5599/admet.4.1.276.
 64. Liu, M.; Yu, J.; Yang, Y.; Tang, K. Deep learning under mass-to-charge ratio pre-retrieval to realize electron ionization mass spectrometry library retrieval. *Rapid Commun Mass Spectrom* **2022**, *36*, e9398, doi:10.1002/rcm.9398.
 65. Stevens, K.G.; Pukala, T.L. Conjugating immunoassays to mass spectrometry: Solutions to contemporary challenges in clinical diagnostics. *Trends Analyt Chem* **2020**, *132*, 116064, doi:10.1016/j.trac.2020.116064.
 66. Xu, S.; Liu, H.; Bai, Y. Highly sensitive and multiplexed mass spectrometric immunoassay techniques and clinical applications. *Anal Bioanal Chem* **2022**, *414*, 5121-5138, doi:10.1007/s00216-022-03945-4.
 67. Trenchevska, O.; Nelson, R.W.; Nedelkov, D. Mass spectrometric immunoassays for discovery, screening and quantification of clinically relevant proteoforms. *Bioanalysis* **2016**, *8*, 1623-1633, doi:10.4155/bio-2016-0060.
 68. Levenson, R.M.; Borowsky, A.D.; Angelo, M. Immunohistochemistry and mass spectrometry for highly multiplexed cellular molecular imaging. *Lab Invest* **2015**, *95*, 397-405, doi:10.1038/labinvest.2015.2.
 69. Salvagno, G.L.; Danese, E.; Lippi, G. Mass spectrometry and total laboratory automation: opportunities and drawbacks. *Clin Chem Lab Med* **2020**, *58*, 994-1001, doi:10.1515/cclm-2019-0723.
 70. DelGuidice, C.E.; Ismaiel, O.A.; Mylott, W.R., Jr.; Halquist, M.S. Comparison of methods for quantitative analysis of ranibizumab and bevacizumab in human plasma using various bioanalytical techniques, including microfluidic immunoassay, triple quadrupole, and high-resolution liquid chromatography-tandem mass spectrometry approaches. *J Pharm Biomed Anal* **2022**, *217*, 114823, doi:10.1016/j.jpba.2022.114823.
 71. El-Khoury, J. Financial considerations for purchasing a mass spectrometer. *MSACL Presentation* **2018**.

-
72. Padula, W.V.; Lee, K.K.H.; Pronovost, P.J. Using Economic Evaluation to Illustrate Value of Care for Improving Patient Safety and Quality: Choosing the Right Method. *J Patient Saf* **2021**, *17*, e568-e574, doi:10.1097/PTS.0000000000000410.
73. Jarosz, M.; Keppler, B.K.; Timerbaev, A.R. Current and emerging mass spectrometry methods for the preclinical development of metal-based drugs: a critical appraisal. *Anal Bioanal Chem* **2022**, *414*, 95-102, doi:10.1007/s00216-021-03718-5.
74. Matheson, A.; Botcherby, L. Trends and Developments in GC and GC-MS: A panel discussion on the latest advances and future developments in gas chromatography mass spectrometry (GC-MS). *The Column* **2020**, *16*, 27-32.
75. Bonnabry, P.; Francois, O. Return on investment: a practical calculation tool to convince your institution. *Eur J Hosp Pharm* **2020**, *27*, 111-113, doi:10.1136/ejhpharm-2018-001733.
76. Lee, J.-G.; Lee, D.-J.; Ri, P.; Kim, K.-T.; Park, S.-J. Case study: An assessment of the economic service life of research equipment in the Korean public research institutes. *The Engineering Economist* **2022**, *67*, 131-156.
77. Giri, C.; Cleaves, H.J.; Meringer, M.; Chandru, K. The Post-COVID-19 Era: Interdisciplinary Demands of Contagion Surveillance Mass Spectrometry for Future Pandemics. *Sustainability* **2021**, *13*, 7614, doi:ARTN 7614
10.3390/su13147614.
78. SoRelle, J.A.; Patel, K.; Filkins, L.; Park, J.Y. Mass Spectrometry for COVID-19. *Clin Chem* **2020**, *66*, 1367-1368, doi:10.1093/clinchem/hvaa222.
79. Popescu, L.; Robu, A.C.; Zamfir, A.D. Sustainable Nanosystem Development for Mass Spectrometry: Applications in Proteomics and Glycomics. *Sustainable Nanosystems Development, Properties, and Applications* **2017**, 535-568, doi:10.4018/978-1-5225-0492-4.ch014.
80. Huss, A.; Peters, A.; Zhao, T.; Barouki, R.; Kogevinas, M.; Vermeulen, R.; Matthies-Wiesler, F. Setting the European environment and health research agenda -under-researched areas and solution-oriented research. *Environ Int* **2022**, *163*, 107202, doi:10.1016/j.envint.2022.107202.
81. Flamini, A.; Jahanshahi, B.; Mohaddes, K. Illegal drugs and public corruption: Crack based evidence from California. *European Journal of Political Economy* **2021**, *69*, 102005, doi:ARTN 102005
10.1016/j.ejpoleco.2021.102005.
82. Varga, Z.; Wallenius, M.; Krachler, M.; Rauff-Nisthar, N.; Fongaro, L.; Knott, A.; Nicholl, A.; Mayer, K. Trends and perspectives in Nuclear Forensic Science. *Trac-Trends in Analytical Chemistry* **2022**, *146*, 116503, doi:ARTN 116503
10.1016/j.trac.2021.116503.
83. Hayashi, H.; Sasajima, H.; Takayanagi, Y.; Kanamaru, H. International standardization for smarter society in the field of measurement, control and automation. In Proceedings of the 2017 56th Annual Conference of the Society of Instrument and Control Engineers of Japan (SICE), 2017; pp. 263-266.
84. De Cremer, D.; Narayanan, D.; Deppeler, A.; Nagpal, M.; McGuire, J. The road to a human-centred digital society: opportunities, challenges and responsibilities for humans in the age of machines. *AI Ethics* **2022**, *2*, 579-583, doi:10.1007/s43681-021-00116-6.
85. Chong, Y.; Bae, J.M.; Kang, D.W.; Kim, G.; Han, H.S. Development of quality assurance program for digital pathology by the Korean Society of Pathologists. *J Pathol Transl Med* **2022**, *56*, 370-382, doi:10.4132/jptm.2022.09.30.

-
86. Al-Kahtani, N.; Alruwaie, S.; Al-Zahrani, B.M.; Abumadini, R.A.; Aljaafary, A.; Hariri, B.; Alissa, K.; Alakrawi, Z.; Alumran, A. Digital health transformation in Saudi Arabia: A cross-sectional analysis using Healthcare Information and Management Systems Society' digital health indicators. *Digit Health* **2022**, *8*, 20552076221117742, doi:10.1177/20552076221117742.
 87. Pandey, D.; Ogunmola, G.A.; Enbeyle, W.; Abdullahi, M.; Pandey, B.K.; Pramanik, S. COVID-19: A Framework for Effective Delivering of Online Classes During Lockdown. *Human Arenas* **2022**, *5*, 322-336, doi:10.1007/s42087-020-00175-x.
 88. Kumar, P.R.; Ravindranath, K.V.; Srilatha, V.; Alobaoid, M.A.; Kulkarni, M.M.; Mathew, T.; Tiwari, H.D. Analysis of Advances in Research Trends in Robotic and Digital Dentistry: An Original Research. *J Pharm Bioallied Sci* **2022**, *14*, S185-S187, doi:10.4103/jpbs.jpbs_59_22.
 89. Brodny, J.; Tutak, M. Assessing the level of digitalization and robotization in the enterprises of the European Union Member States. *PLoS One* **2021**, *16*, e0254993, doi:10.1371/journal.pone.0254993.
 90. Garard, J.; Wood, S.L.R.; Sabet-Kassouf, N.; Ventimiglia, A.; Matthews, H.D.; Ubalijoro, E.; Chaudhari, K.; Ivanova, M.; Luers, A.L. Moderate support for the use of digital tracking to support climate-mitigation strategies. *One Earth* **2022**, *5*, 1030-1041, doi:10.1016/j.oneear.2022.08.005.
 91. Jarockyte, G.; Karabanovas, V.; Rotomskis, R.; Mobasheri, A. Multiplexed Nanobiosensors: Current Trends in Early Diagnostics. *Sensors (Basel)* **2020**, *20*, doi:10.3390/s20236890.
 92. Gil Rosa, B.; Akingbade, O.E.; Guo, X.; Gonzalez-Macia, L.; Crone, M.A.; Cameron, L.P.; Freemont, P.; Choy, K.L.; Guder, F.; Yeatman, E.; et al. Multiplexed immunosensors for point-of-care diagnostic applications. *Biosens Bioelectron* **2022**, *203*, 114050, doi:10.1016/j.bios.2022.114050.
 93. Vukajlović, J.M.; Panić-Janković, T. Mass Spectrometry in Clinical Laboratories. In *Mass Spectrometry in Life Sciences and Clinical Laboratory*; IntechOpen: 2021.
 94. Kitata, R.B.; Yang, J.C.; Chen, Y.J. Advances in data-independent acquisition mass spectrometry towards comprehensive digital proteome landscape. *Mass Spectrom Rev* **2022**, e21781, doi:10.1002/mas.21781.
 95. Carayannis, E.G.; Morawska-Jancelewicz, J. The Futures of Europe: Society 5.0 and Industry 5.0 as Driving Forces of Future Universities. *Journal of the Knowledge Economy* **2022**, *13*, 3445-3471, doi:10.1007/s13132-021-00854-2.
 96. Khare, S.; Chourasia, U.; Deen, A.J. Load balancing in cloud computing. In Proceedings of the Proceedings of the International Conference on Cognitive and Intelligent Computing, 2022; pp. 601-608.
 97. Soete, M.; De Bruycker, K.; Du Prez, F. Rewritable Macromolecular Data Storage with Automated Read-out. *Angew Chem Int Ed Engl* **2022**, *61*, e202116718, doi:10.1002/anie.202116718.
 98. de Koning, S.; Janssen, H.-G.; Brinkman, U.A.T. Modern methods of sample preparation for GC analysis. *Chromatographia* **2009**, *69*, 33-78.
 99. Falaki, F. Sample preparation techniques for gas chromatography-gas chromatography-derivatization, sample preparation, application. *London, United Kingdom: IntechOpen* **2019**, *10*.
 100. Smith, P.A.; Lepage, C.J.; Lukacs, M.; Martin, N.; Shufutinsky, A.; Savage, P.B. Field-portable gas chromatography with transmission quadrupole and cylindrical ion trap mass spectrometric detection: Chromatographic retention index data and ion/molecule interactions for chemical warfare agent identification. *International Journal of Mass Spectrometry* **2010**, *295*, 113-118, doi:10.1016/j.ijms.2010.03.001.

101. Xu, Y.; Liu, X.; Cao, X.; Huang, C.; Liu, E.; Qian, S.; Liu, X.; Wu, Y.; Dong, F.; Qiu, C.W.; et al. Artificial intelligence: A powerful paradigm for scientific research. *Innovation (Camb)* **2021**, *2*, 100179, doi:10.1016/j.xinn.2021.100179.
102. Yang, X.; Wang, Y.; Byrne, R.; Schneider, G.; Yang, S. Concepts of Artificial Intelligence for Computer-Assisted Drug Discovery. *Chem Rev* **2019**, *119*, 10520-10594, doi:10.1021/acs.chemrev.8b00728.
103. Agatonovic-Kustrin, S.; Beresford, R. Basic concepts of artificial neural network (ANN) modeling and its application in pharmaceutical research. *J Pharm Biomed Anal* **2000**, *22*, 717-727, doi:10.1016/s0731-7085(99)00272-1.
104. Jaillant, L.; Caputo, A. Unlocking digital archives: cross-disciplinary perspectives on AI and born-digital data. *AI Soc* **2022**, *37*, 823-835, doi:10.1007/s00146-021-01367-x.
105. Ries, T.; Palkó, G. Born-digital archives. **2019**, *1*, 1-11.
106. Sergi, C.M. Digital pathology: the time is now to bridge the gap between medicine and technological singularity. In *Interactive Multimedia-Multimedia Production and Digital Storytelling*; IntechOpen London, UK: 2019.
107. Ahmed Alaa El-Din, E. ARTIFICIAL INTELLIGENCE IN FORENSIC SCIENCE: INVASION OR REVOLUTION? *Egyptian Society of Clinical Toxicology Journal* **2022**, *10*, 20-32.
108. Jones, A. Biochemical and physiological research on the disposition and fate of ethanol in the body. *Medico legal aspects of alcohol, 5th edition, Lawyers and Judges publishing company, Tucson* **2008**.
109. Jones, A.W. Driving under the influence of alcohol. *Handbook of Forensic Medicine* **2022**, *3*, 1387-1408.
110. Katz, P.S. Expert Robot: Using Artificial Intelligence to Assist Judges in Admitting Scientific Expert Testimony. *Albany Law Journal of Science & Technology* **2014**, *24*.
111. Whitford, A.B.; Yates, J.; Burchfield, A.; Anastasopoulos, J.L.; Anderson, D.M. The Adoption of Robotics by Government Agencies: Evidence from Crime Labs. *Public Administration Review* **2020**, *80*, 976-988, doi:10.1111/puar.13301.
112. Martins, C.; Dreij, K.; Costa, P.M. The State-of-the Art of Environmental Toxicogenomics: Challenges and Perspectives of "Omics" Approaches Directed to Toxicant Mixtures. *Int J Environ Res Public Health* **2019**, *16*, doi:10.3390/ijerph16234718.
113. Quireyns, M.; Boogaerts, T.; Van Wichelen, N.; Covaci, A.; Van Nuijs, A.L.N. State-of-the-art analytical approaches and strategies to assess disposal of drugs for wastewater-based epidemiology. *Wiley Interdisciplinary Reviews: Forensic Science* **2022**, e1469, doi:10.1002/wfs2.1469.
114. Pélissier-Alicot, A.-L. Synthetic cannabinoids: State-of-the-art with a focus on fertility and development. In *Cannabis and the Developing Brain*; Elsevier: 2022; pp. 243-258.
115. Aggarwal, K.; Mijwil, M.M.; Al-Mistarehi, A.-H.; Alomari, S.; Gök, M.; Alaabdin, A.M.Z.; Abdulrhman, S.H. Has the Future Started? The Current Growth of Artificial Intelligence, Machine Learning, and Deep Learning. *Iraqi Journal for Computer Science and Mathematics* **2022**, *3*, 115-123.
116. Svensson, A.M.; Jotterand, F. Doctor ex machina: A critical assessment of the use of artificial intelligence in health care. In *Proceedings of the The Journal of Medicine and Philosophy: A Forum for Bioethics and Philosophy of Medicine, 2022*; pp. 155-178.
117. Whiting, P.F.; Rutjes, A.W.; Westwood, M.E.; Mallett, S.; Deeks, J.J.; Reitsma, J.B.; Leeflang, M.M.; Sterne, J.A.; Bossuyt, P.M.; Group, Q.-. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* **2011**, *155*, 529-536, doi:10.7326/0003-4819-155-8-201110180-00009.

-
118. Pantic, I.; Paunovic, J.; Cumic, J.; Valjarevic, S.; Petroianu, G.A.; Corridon, P.R. Artificial neural networks in contemporary toxicology research. *Chem Biol Interact* **2023**, *369*, 110269, doi:10.1016/j.cbi.2022.110269.
 119. Mehrvar, S.; Himmel, L.E.; Babburi, P.; Goldberg, A.L.; Guffroy, M.; Janardhan, K.; Krempley, A.L.; Bawa, B. Deep Learning Approaches and Applications in Toxicologic Histopathology: Current Status and Future Perspectives. *J Pathol Inform* **2021**, *12*, 42, doi:10.4103/jpi.jpi_36_21.
 120. Raju, B.; Jumah, F.; Ashraf, O.; Narayan, V.; Gupta, G.; Sun, H.; Hilden, P.; Nanda, A. Big data, machine learning, and artificial intelligence: a field guide for neurosurgeons. *J Neurosurg* **2020**, *1*, 1-11, doi:10.3171/2020.5.JNS201288.

