Poznan University of Medical Sciences
Poland

JMS Journal of Medical Science

previously Nowiny Lekarskie

Founded in 1889

2022
Vol. 91, No. 2

QUARTERLY

Indexed in:
DOAJ, Crossref, Google Scholar,
Polish Medical Bibliography, Index Copernicus,
Ministry of Education and Science

eISSN 2353-9801
ISSN 2353-9798

www.jms.ump.edu.pl

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10 Fredry Street, 61-701 Poznań, Poland
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www.ump.edu.pl

eISSN 2353-9801
ISSN 2353-9798
DOI: 10.20883/ISSN.2353-9798

Publishing Manager: Grażyna Dromirecka
Technical Editor: Bartłomiej Wąsiel

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Ark. wyd. 8,0. Ark. druk. 7,5.
Zam. nr 33/22.

The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

You are welcome to publish your basic, medical and pharmaceutical science articles in *Journal of Medical Science*.

Ethical guidelines

The Journal of Medical Science applies the ethical principles and procedures recommended by COPE (Committee on Conduct Ethics), contained in the Code of Conduct and Best Practice Guidelines for Journal Editors, Peer Reviewers and Authors available on the COPE website: https://publicationethics.org/resources/guidelines

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REVIEW PAPER

JMS Journal of Medical Science

INVITED EDITORIAL

Publication ethics of human studies in the light of the Declaration of Helsinki – a mini-review

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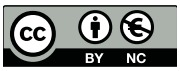
DOI: https://doi.org/10.20883/medical.e700

Keywords: ethics, Declaration of Helsinki, publication, medical research

Published: 2022-06-30

How to Cite: Sawicka-Gutaj N, Gruszczyński D, Guzik P, Mostowska A, Walkowiak J. Publication ethics of human studies in the light of the Declaration of Helsinki – a mini-review. 2022;91(2);e700. doi:10.20883/medical.e700



ABSTRACT

The Declaration of Helsinki is a set of ethical principles to be followed by scientists involved in medical research with humans or human cells and tissues. This Declaration defines how scientific research should be planned, conducted, documented, analysed, and published.

We summarise and discuss some ethical issues related to publishing original articles, including clinical trials, review papers, and case reports based on the seventh revision of the Declaration of Helsinki.

The principles of the Declaration of Helsinki refer primarily to the publication of medical research results, in particular clinical trials, as original articles. Such papers are required to meet several ethical requirements, particularly the study protocol transparency and the presentation of the results. In terms of case reports, the bioethical aspects related to their publication are twofold - they need to include informed and voluntary consent and the confidentiality of study participants. The review papers are of the least bioethical concern. However, whether patients' agreements with specific studies are valid if the data are used in meta-analyses is uncertain.

Adherence to ethical policies and standards helps to ensure the highest possible quality of scientific publications. Responsibility for compliance with the Declaration of Helsinki lies not only with the authors preparing their manuscripts, but also with the editorial board and reviewers, who must evaluate the ethical soundness of the submitted papers. The additional guidelines for the different types of studies facilitate the implementation of the Declaration principles.

Introduction

The Declaration of Helsinki is a fundamental document establishing principles for conducting scientific research involving humans [1]. Following the introduction of the Nuremberg Code in 1947, the World Medical Association (WMA) published the first version of the Declaration in 1964. The Nuremberg Code and the Declaration of Helsinki defined the legal principles for conducting medical experiments on humans for the first time.

Since its announcement, the Declaration of Helsinki has been improved and changed seven times. The latest version from 2013 is now in force [1]. In addition to the WMA official languages (English, Spanish, French), this document is also available in other languages, e.g. Polish, German, and Japanese.

The Declaration of Helsinki – seventh revision [2]

The preamble is addressed primarily to physicians. However, it is recommended that its contents be shared with other members of research teams involved in human medical studies. The same applies to research teams with no physicians, such as dieticians, psychologists, physiotherapists, coaches etc.

The 7th version of the Declaration of Helsinki introduces the new term. i.e. "medical research" to reflect all scientific medical studies on human material. Previous versions related the term "research" directly only to medical experiments on humans. It consists of 37 paragraphs describing various ethical issues and regulating conducting medical research.

Medical research involving humans and human biological material (e.g., cells, tissues) should be performed only by individuals with appropriate ethical and scientific education, training and qualifications (paragraph 12). Scientific aims to gain new insights and gather interesting data should not be superior to the rights of study participants by any member of medical research teams (paragraph 8).

Physicians who are researchers have various duties. In particular, they need to protect health and life, well-being and patients' rights, such as dignity, integrity, self-determination, privacy and confidentiality of personal data (paragraphs 4, 9 and 24). Each scientist must assess the study participants' risk, burden, and benefits to minimise the adverse effects of investigated interventions or employed methods. Participants' activities must be precisely and conscientiously monitored (paragraph 17). Each study participant or legal representative of such a person (e.g. unconscious or under-age or mentally disabled) must receive necessary information regarding the study protocol and forms. Informed and voluntary consent must be collected from all participants, or their legal representatives, preferably in writing (paragraphs 25-26). Similarly, in terms of research on human material stored in biobanks for re-use, informed consent from the donor is required (paragraph 32).

The Declaration of Helsinki considers the procedure in exceptional situations, including studies on vulnerable humans, the use of placebo and interventions with unproven efficacy (paragraphs 19, 33 and 37). Following the completion of clinical trials, it describes steps to be followed (paragraph 34).

Particular attention should be paid to the protocols concerning the design and conduct of medical research in light of the applicable bioethical principles. All study protocols must be submitted, reviewed and approved by an independent and competent local or regional bioethics committee (paragraph 23). Medical research involving humans must be designed and conducted strictly according to previous protocols. Developing each study protocol should be preceded by a thorough analysis of the scientific literature concerning specific topics (paragraphs 21-22). In this way, unnecessary repetitive studies concluded by "rediscovering" the already known facts can be avoided. Another crucial issue is the complete and reliable dissemination of research results. Ethical scientists should not select only some positive results which confirm their hypotheses. Publication of research results different from the assumed and anticipated, negative, or demonstrating harmful effects of interventions should be obligatory. The concealment of adverse or ambiguous study outcomes is always deliberate, and selective non-transparency is tantamount to scientific manipulation. Funding sources, institutional links and conflicts of interest must always be disclosed (paragraph 36).

Adherence to ethical policies and standards helps to ensure the highest possible quality of scientific publications. Responsibility for compliance with the Declaration of Helsinki lies not only with the authors preparing their manuscripts, but also with the editorial board and reviewers, who also need to evaluate the ethical soundness of the submitted papers. Scientific reports of original studies, review papers (including systematic reviews), and case reports which have not been prepared in accordance with the principles of the Declaration of Helsinki should not be accepted for publication.

Original articles

The Declaration of Helsinki is most relevant to original articles directly presenting the results of medical research involving humans and human material. Regulations of this Declaration also address clinical trials.

Following the Declaration of Helsinki rules is essential throughout the entire scientific process. Its regulations must be considered during the planning stage while conducting the designed investigation and disseminating the obtained results. Authors are required to report a detailed study protocol (to allow replication of the study by other researchers), with the prior approval of which the appropriate bioethical committee.

Both the details of the research methodology and protocols, as well asthe clarity of the results are important. Authors should present the results completely, not concealing ambiguous results that can undermine the assumed conclusions. The Declaration of Helsinki emphasises the transparency of clinical trials through their obligatory registration – it reduces publication and reporting bias and provides reliable evidence for decision-making [3]. Moreover, the 7th version of the Declaration extensively discusses and pays special attention to the use of placebo, or other interventions with unproven efficacy, assessment of the risks and benefits of the study, compensation for potential harm to study participants, and treatment continuation following the clinical trial completion. The authors should precisely describe all the above aspects [4,5]. It is also necessary to include statements about obtaining informed consent from study participants, conflicts of interest, and research funding sources [6].

The Consolidated Standards of Reporting Trials (CONSORT) statement has been developed to improve the quality of clinical trial reporting. The checklist has been prepared to increase the transparency and completeness of research protocols. In fact, the included items comprise recruitment criteria and flow, type of randomisation and blinding, or sample size justification. These guidelines emphasise the need to discuss the study limitations, considering potential bias sources and the generalisation of the obtained findings for their applicability in clinical practice [7]. Similarly, the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and the Standards for Reporting of Diagnostic Accuracy Studies (STARD) statements have been developed for other types of research involving human subjects [8, 9].

Case reports

For the publication of clinical case reports, two bioethical issues are essential in view of the Declaration of Helsinki. Firstly, obtaining informed and voluntary consent, and secondly, securing the confidentiality of research participants.

The patient presented must be fully informed regarding the publication and its content, including the extent of the patient's medical data. Prior to the publication, the patient's consent must be obtained, based on the information provided to him/her previously. In case of underage, unconscious, or mentally disabled patients, this consent must be obtained from their guardian or legal representative. Prior to the manuscript submission, it is also advisable to provide the prepared material to the patient or the caregiver for authorisation. Some journals require written approval from the patient to publish such a paper [10].

Obtaining the authorisation entails the obligation to verify the published medical data (such as photos, imaging studies, etc.) to maintain the privacy of the presented persons. In order to protect the patient's confidentiality, all personal data and data identifying the patient should be removed from the case report. It is vital for people living in small communities where detailed information about their medical or family history may allow their identity to be established [11].

Importantly, both of the mentioned issues are included in the CAse REport (CARE) guidelines checklist (item 5a – "De-identified patient specific information", and item 13 – "Did the patient give informed consent? Please provide if requested") [12]. Case reports should contain all the necessary detailed data, including de-identified patient-specific information, concerns and symptoms, medical and family history, significant clinical examination findings, diagnostic evaluation and administered therapeutic intervention [13]. Transparently written case reports provide sufficient information for clinical research, allow the creation of clinical practice guidelines and improve medical education [14].

Review papers

In contrast to both original and case reports, review papers usually raise minor ethical concerns regarding the Declaration of Helsinki. The preparation of review papers does not require an opinion from the competent bioethics committee. The selection of the cited articles depends solely on the authors of the prepared review. They are responsible for ensuring the reliability and scientific soundness without raising ethical doubts.

In terms of the systematic reviews, their preparation and structure are strictly defined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines [15]. Furthermore, authors have more freedom in the manuscript form for the narrative reviews. Systematic reviews may include a qualitative and quantitative analysis of the cited papers. The authors are responsible for formulating the objective, criteria for inclusion and exclusion of the articles, and assessing the risk of bias and evidence level.

The question arises to what extent the aim set by the authors of the meta-analysis should be comparable to the aims of the analysed original studies [16]. Participants are informed about the specific objectives and methods of specific studies, and it is uncertain whether their informed and voluntary consent can be extrapolated to other, not primarily planned, investigations, such as meta-analysis. Moreover, it is disputable if initial consent can and should be implied for future research by other authors.

The latest guidelines for reporting systematic reviews [15] also include the need to register the reviews which are prepared together with their protocols to avoid duplication of projects and increase their transparency. It is in line with the Declaration of Helsinki recommendation on the registration of medical research. Additionally, the authors of review papers are obliged to declare the financial and non-financial support sources for the review, as well ascompeting interests. It is also recommended to discuss the limitations of each review and its implications for clinical practice or further research.

As in the PRISMA guidelines, the criteria for assessing the quality of the included original papers practically do not consider the ethical evaluation of the conducted studies. Instead, they focus on methodological issues, such assample size, homogeneity of the study participants, and appropriate matching of the control group. It is difficult to assess, usually with hindsight, the ethical issues of the previously conducted research for multiple reasons. These include the demographic and ethnic diversity of the study participants and researchers, as well as the variability in time, place, and standards of conduct [16].

Conclusions

When publishing scientific papers on research involving human subjects and human material, the recommendations from the Declaration of Helsinki have to be respected. Responsibility for the compliance with this Declaration lies with the authors preparing the manuscripts, the members of the editorial boards developing the criteria for publication of papers in their journals, and the reviewers assessing the substantive and ethical value of the presented findings. Is is clear that the additional guidelines for the different types of studies facilitate the implementation of the Declaration principles and expand the range of issues covered in the field of research protocols.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

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ORIGINAL PAPER

JMS Journal of Medical Science

A comparison of postoperative blood lactate concentrations and kinetics in cardiac surgical patients receiving and not receiving metformin

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Keywords: Metformin, Lactate, Lactate clearance, cardiac surgery

Published: 2022-07-11

How to Cite: Ampatzidou F, Diplaris K, Drosos O, Drossos G. A comparison of postoperative blood lactate concentrations and kinetics in cardiac surgical patients being and not being administered metformin. Journal of Medical Science. 2022;91(2);e631. doi:10.20883/medical.e631

DOI: https://doi.org/10.20883/medical.e631

ABSTRACT

Aim. Early discontinuation of metformin before cardiac surgery is advised by several national societies, although no hard evidence exist supporting this practice. This precaution is mostly extrapolated by data on different clinical settings. The aim of this study is to investigate the impact of preoperative metformin use on lactate concentrations and lactate clearance during the first postoperative day following cardiac surgery.

Material and Methods. Among 367 consecutive patients who underwent elective on-pump cardiac surgery from January 2019 to October 2019, 109 were diabetics, 74 of whom were treated with metformin. Data on lactate concentrations and clearance during the first postoperative day were prospectively collected on arrival in the ICU, as well as after H6, H12 and H24 in the ICU and subsequently compared. A subgroup analysis focusing only on the diabetic patients was also performed. Repeated measures multivariate analysis of variance (MANOVA) was used to investigate the data on the basis of the group, time and their interaction effects.

Results. Lactate concentrations were the same for both groups upon arrival in the ICU. Interestingly, metformin users presented lower lactate concentrations than non-users on the following measurements ($p = 0.003$ at 6 h and $p = 0.01$ at 24 h). No significant interaction was found between the two groups ($p = 0.76$). No difference was found between the two groups in terms of lactate clearance ($p = 0.53$). In the subgroup analysis no difference was observed between metformin users and non-users, either on lactate concentrations ($p = 0.61$), or on lactate clearance ($p = 0.86$).

Conclusions. In the intensive care unit setting following heart surgery, the use of metformin up until the night before surgery was not associated with increased postoperative lactate concentrations or impaired lactate clearance.

Introduction

Metformin is a biguanide with excellent properties in terms of glycemic control which makes it a first choice oral agent for the treatment of patients with diabetes mellitus [1]. However, conflicting data exist regarding its impact on lactate kinetics [2, 3]. Moreover, its use has been associated with the Metformin Associated Lactic Acidosis (MALA), a rare, but potentially fatal complication, with a nearly 50% mortality rate, occurring mostly in patients with preexisting renal, cardiac or hepatic dysfunction [4].

Additionally, MALA is more common in metformin users with concomitant acute pathologic conditions, such as sepsis, acute liver dysfunction, tissue hypoperfusion, or acute kidney dysfunction due to hypovolemia.

This potential threat has lead several national societies and institutions to recommend its discontinuation prior to surgery [5–7].

Lactate and lactate elimination rate have a prognostic value in cardiac surgical patients [8, 9]. In this setting, however, hyperlactatemia could be due to either excess lactate production due to tissue hypoperfusion, or a decreased rate of lactate metabolism and clearance. High lactate concentration is an indicator of hemodynamic dysfunction. If metformin leads to an increase in lactate levels, it may be misinterpreted as hemodynamic compromise which should be rapidly corrected, particularly in this group of patients. This may result in unnecessary fluid resuscitation, or in the administration of inotropes. To this point no guidelines on the perioperative use of metformin have been issued by the European, or the American Associations of Anesthesiology. In our department, treatment with metformin is discontinued only on the day of the surgery, and it is restarted 48 hours postoperatively.

Aim

The aim of this study is to investigate whether the continued use of metformin prior to elective cardiac surgery has any impact on lactate concentrations and clearance during the early postoperative period.

Material and Methods

Study Design

In our department, 367 consecutive patients underwent elective on-pump cardiac surgery from January 2019 to October 2019. This group included 109 patients with diabetes mellitus, 74 of whom were treated with metformin. The rest of the diabetic patients (35 patients) were treated either with different oral agents, or with insulin. In our institution, we do not discontinue metformin prior to surgery in diabetic patients who use it to control their blood glucose levels.

For the purpose of this study, we compared postoperative blood lactate concentrations in patients using metformin (n = 74) and those of non-users (n = 293). A subgroup analysis focusing on the diabetic patients (n = 109) was also conducted to control for diabetes status as a possible confounder. Demographic and perioperative characteristics were recorded for the entire cohort.

In all patients, arterial blood samples were taken postoperatively on 4 time points: arrival in the ICU, H6, H12 and H24 after ICU arrival. Lactate concentration in mmol/L was measured in these samples with a Radiometer ABL800 FLEX point-of-care whole blood analyzer. As epinephrine may elevate lactate concentration without tissue hypoperfusion, its dose of continuous administration in μγ/Kg/min was recorded at the same time points. In order to exclude potential liver failure, causing a low lactate metabolism, the maximum values of liver enzymes, aspartate (AST) and alanine (ALT) transaminases, were recorded for each patient. Lactate concentrations and lactate clearance were compared between groups over time.

Lactate clearance was calculated using the following formula:

$$\frac{Lac\ ar - Lac\ t}{Lac\ ar}$$

where Lac ar. stands for the lactate concentration measured on arrival in the ICU and Lac_t for the lactate concentration at a specific time point (t).

Lactate clearance as defined here is an indicator of lactate elimination rate, and does not represent a pharmacokinetic parameter.

A positive value indicates a decrease in lactate blood concentration over time and, hence, its

clearance from the body, whereas a negative value indicates the inability to clear lactate from the blood circulation.

Statistical Analysis

Descriptive statistics were used to summarize patient characteristics. Continuous variables are reported as means and standard deviation, whereas categorical values as counts and percentages. Comparisons between groups were performed using Wilcoxon signed-rank test for continuous variables, while comparisons of categorical variables were performed by means of the Chi-square test, or Fisher's exact test for extreme proportions, as appropriate. Statistical tests were based on a two-sided significance level of 0.05. The repeated measures multivariate analysis of variance (MANOVA) was performed to investigate the group, time and their interaction effects.

The SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA) was used to perform statistical analyses. Any potential outlier was included in the analysis and no imputation methods were considered.

Results

Baseline characteristics and perioperative parameters for the entire cohort are shown in **Table 1**. Preoperative parameters did not differ between the two groups. Metformin users had a significantly shorter CPB time by a mean of 13 minutes. Data regarding lactate measurements are shown in **Table 2**. Lactate concentrations were the same for both groups upon arrival in the ICU. Interestingly, metformin users presented lower concentrations of lactates than non-users in terms of the following measurements.

Figure 1 shows lactate concentrations for the two patient groups over time. In both groups, lactate values significantly decreased over time indicating a good clearance from the body. The interaction between groups was not significant. As shown in **Figure 2** lactate clearance increased over time, although no significant difference was found between the two groups on MANOVA. (**Table 3**).

A subgroup analysis including only patients with diabetes mellitus was also performed. Pre and perioperative characteristics of these patients

Table 1. Demographics and baseline characteristics of patients on the entire cohort analysis

	All patients (n = 367)	Metformin users (n = 74)	Non metformin users (n = 293)	p-value
Age (y)	66.5 ± 10.0	67.0 ± 8.4	66.4 ± 10.4	0.933
Female gender(%)	67 (18.3%)	10 (13.5%)	57 (19.5%)	0.237
EuroScore II	2.0 ± 2.4	1.6 ± 1.2	2.1 ± 2.6	0.292
Body Mass Index	28.7 ± 4.6	29.1 ± 4.8	28.6 ± 4.5	0.481
Smokers	130 (35.4%)	27 (36.5%)	103 35.2%)	0.830
GFR	79.7 ± 24.8	80.9 ± 24.9	79.4 ± 24.8	0.885
GFR < 60ml/min	73 (19.9%)	16 (21.6%)	57 (19.5%)	0.676
Peripheral Vascular Disease	65 (17.7%)	16 (21.6%)	49 (16.7%)	0.324
Chronic Obstructive Pulmonary Disease	33 (9.0%)	10 (13.5%)	23 (7.8%)	0.123
NYHA status:				
1	209 (56.9%)	46 (62.2%)	163 (55.6%)	0.397
2	140 (38.1%)	27 (36.5%)	113 (38.6%)	
3	15 (4.1%)	1 (1.4%)	14 (4.8%)	
4	3 (0.8%)	0 (0%)	3 (1%)	
LV Ejection Fraction	54.0 ± 11.1	52.7 ± 10.9	54.3 ± 11.2	0.262
Bypass Time	105.0 ± 38.8	94.1 ± 39.3	107.7 ± 38.3	0.033

Table 2. Repeated measures Multivariate ANalysis Of VAriance of lactate concentrations in the entire cohort (N = 367)

Lactate concentration (mmol/L)	Metformin users (n = 74)	Non metformin users (n = 293)	p-value	Group Effect	Time Effect	Time*Group Effect
On arrival	3.7 ± 0.2	4.1 ± 0.1	0.2069	0.0338	<0.0001	0.7595
6h	2.9 ± 0.3	3.3 ± 0.1	0.1846			
12h	1.6 ± 0.2	2.1 ± 0.1	0.0035			
24 h	1.3 ± 0.1	1.7 ± 0.1	0.0112			

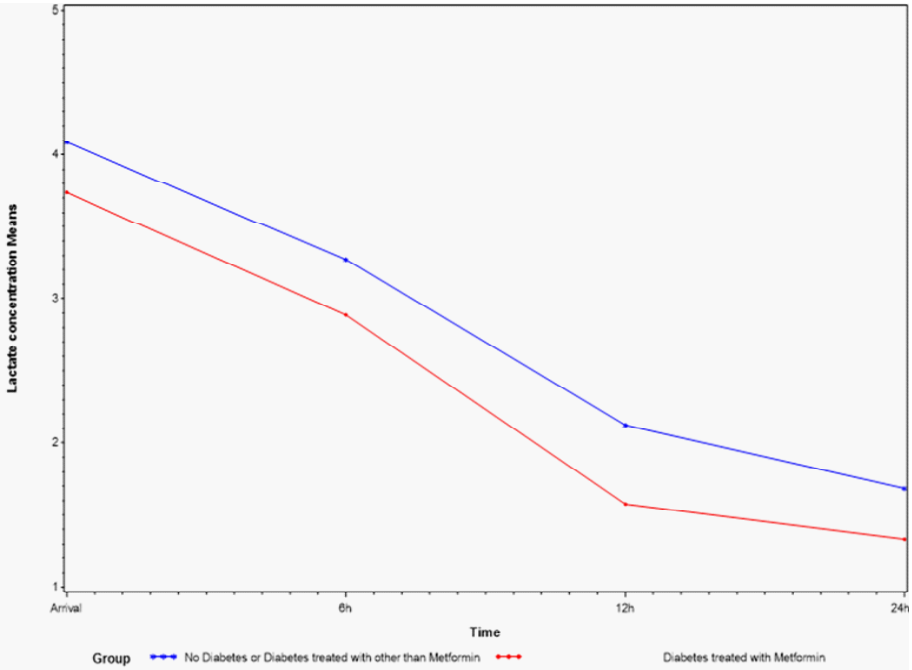

Figure 1. Lactate Concentration over time (all patients). In both groups (diabetics treated with metformin vs. the rest of the cohort), lactate levels significantly decreased over time

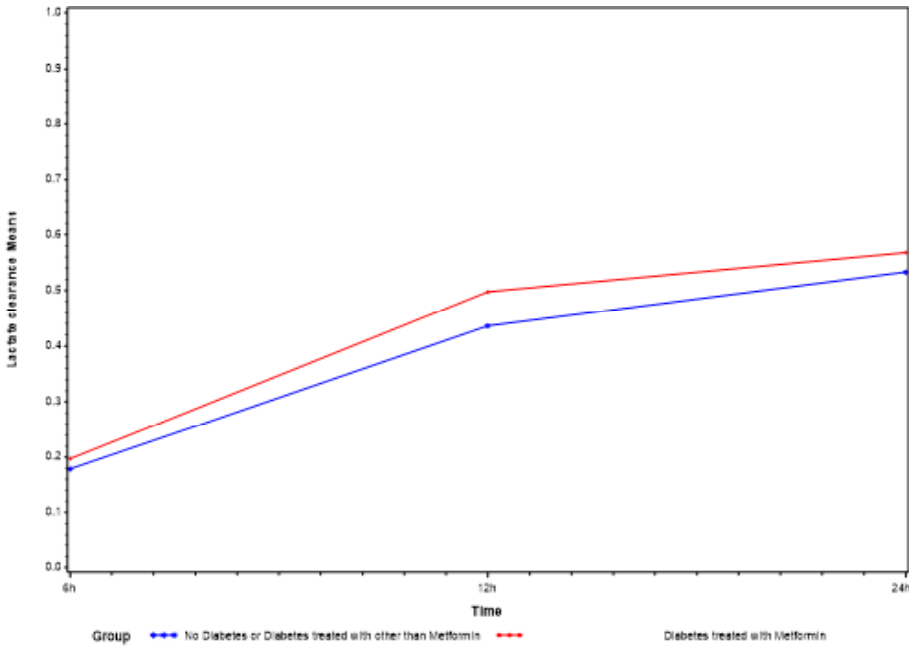

Figure 2. Lactate Clearance over time (all patients). Lactate clearance increased over time, although no significant difference was found between the two groups (diabetics treated with metformin vs. the rest of the cohort)

Table 3. Repeated measures Multivariate ANalysis Of VArianc of lactate clearance in the entire cohort (N = 367)

Lactate clearance	Metformin users (n = 74)	Non metformin users (n = 293)	p-value	Group Effect	Time Effect	Time*Group Effect
6h	0.2	0.2	0.7097	0.2536	<0.0001	0.5292
12h	0.5	0.4	0.1126			
24h	0.6	0.5	0.2552			

are presented in **Table 4**. Metformin users demonstrated a better patient profile with lower Euroscore II and a higher GFR than non-users. These findings were to be expected as insulin dependent diabetics are included in the non-metformin users group, and insulin dependent diabetes mel-

Table 4. Demographics and baseline characteristics of diabetic patients included in the subgroup analysis

	TOTAL (n = 109)	Metformin users (n = 74)	Non Metformin users (n = 35)	p-value
Age (y)	68.1 ± 8.4	67.0 ± 8.4	70.3 ± 8.1	0.059
Female gender	20 (18.3%)	10 (13.5%)	10 (28.6%)	0.058
EuroScore II	2.2 ± 2.5	1.6 ± 1.2	3.3 ± 3.9	0.026
Body Mass Index	28.9 ± 4.8	29.1 ± 4.8	28.4 ± 4.9	0.453
Smokers	35 (32.1%)	27 (36.5%)	8 (22.9%)	0.155
GFR	77.0 ± 25.7	80.9 ± 24.9	68.9 ± 25.8	0.041
GFR < 60ml/min	28 (25.7%)	16 (21.6%)	12 (34.3%)	0.158
Peripheral Vascular Disease	25 (22.9%)	16 (21.6%)	9 (25.7%)	0.635
Chronic Obstructive Pulmonary Disease	16 (14.7%)	10 (13.5%)	16 (17.1%)	0.294
NYHA status:				
1	65 (59.6%)	46 (62.2%)	19 (54.3%)	0.673
2	42 (38.5%)	27 (36.5%)	15 (42.9%)	
3	2 (1.8%)	1 (1.4%)	1 (2.9%)	
4	0 (0%)	0 (0%)	0 (0%)	
Preoperative LV Ejection Fraction	53.1 ± 11.2	52.7 ± 10.9	53.9 ± 11.9	0.619
Bypass time	100.4 ± 43.2	94.1 ± 39.3	113.7 ± 48.4	0.251

Table 5. Repeated measures Multivariate ANalysis Of VAriance of lactate concentrations in the subgroup of diabetic patients (N = 109)

Lactate concentration (mmol/L)	Metformin users (n = 74)	Non metformin users (n = 35)	p-value	Group Effect	Time Effect	Time*Group Effect
On arrival	3.7 ± 0.2	4.3 ± 0.3	0.2061	0.2045	<0.0001	0.6112
6h	2.9 ± 0.2	3.3 ± 0.3	0.3789			
12h	1.6 ± 0.1	1.8 ± 0.2	0.2517			
24h	1.3 ± 0.1	1.4 ± 0.1	0.3523			

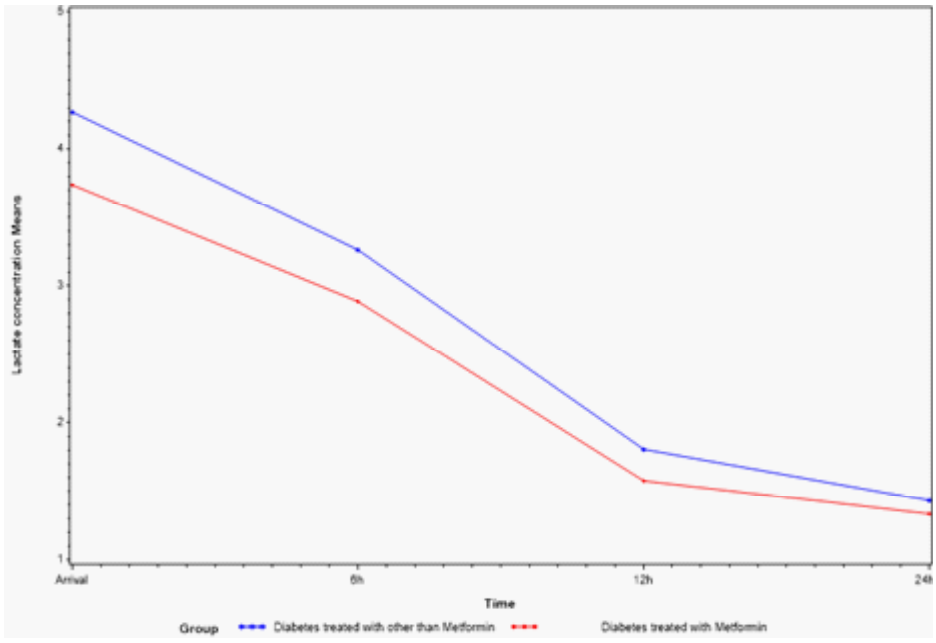

Figure 3. Lactate Concentration over time (diabetics). In both groups (diabetics treated with metformin vs. diabetics treated with other drugs, or insulin), lactate values significantly decreased over time

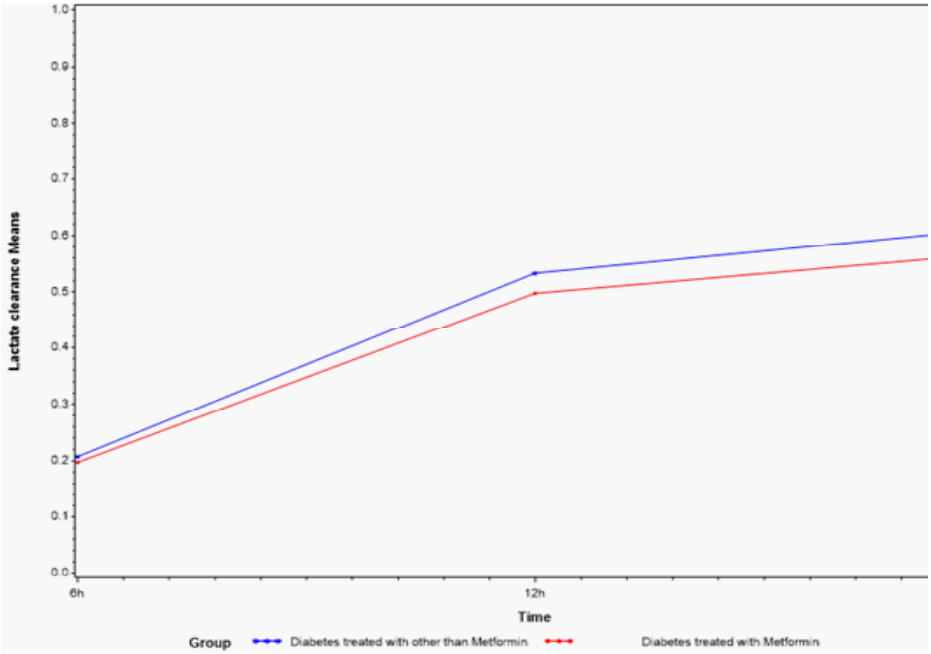

Figure 4. Comparison between 2 groups (diabetics treated with metformin vs. diabetics treated with other drugs, or insulin): lactate clearance increased over time

Table 6. Repeated measures Multivariate ANalysis Of VAriance of lactate clearance in the subgroup of diabetic patients (N = 109)

Lactate clearance	Metformin users (n = 74)	Non metformin users (n = 35)	p-value	Group Effect	Time Effect	Time*Group Effect
6h	0.2	0.2 ± 0.1	0.9093	0.5991	<0.0001	0.8621
12h	0.5	0.5 ± 0.1	0.5850			
24h	0.6	0.6	0.2954			

litus constitutes a known perioperative risk factor. Patients treated with metformin tended also to be mostly male and younger than the rest of the diabetic population; nevertheless, these tendencies did not reach statistical significance.

Table 5 shows data with regard to the lactate measurements for patients of the subgroup analysis. No differences were found between the two groups. Lactate concentrations decreased significantly over time in all patients indicating a good clearance as shown in **Figure 3**. Lactate clearance increased over time in both groups (**Figure 4**). No differences on lactate clearance were found between the two groups of the subgroup analysis (**Table 6**).

Discussion

Hyperlactatemia represents a marker of tissue hypoxia/hypoperfusion mainly through anaerobic glycolysis. Increased blood lactate levels are associated with significant morbidity and mortality in different groups of patients [10–12]. In cardiac surgery, lactate and lactate clearance have prognostic value [8, 9, 12]. Hyperlactatemia can be the result of either excess lactate production due to tissue hypoperfusion, or a decreased rate of lactate metabolism and clearance. However, different physiologic states, such as a stress response, the hyperdynamic stage of sepsis, or β-2 adrenergic receptors stimulation, commonly used in cardiac surgery, may increase lactate formation resulting in transient hyperlactatemia without tissue hypoxia [13, 14].

Metformin enhances tissue sensitivity to insulin, increases peripheral uptake of glucose, decreases glucose absorption in the digestive tract, suppresses neoglycogenesis and decreases fatty acid oxidation, thus making it a first choice oral agent in the treatment of diabetes mellitus in patients with normal kidney and liver func-

tions. However, its use has been associated with impaired lactate kinetics, hyperlactatemia and lactic acidosis [2–4]. MALA is an extremely rare event with an estimated incidence of 0.03 to 0.06 per 1000 patient-years [1, 15], and occurs when there is an imbalance between increased lactate production and impaired metabolism/reduced clearance. Metformin plasma levels >5 μg/mL are generally found when metformin is implicated as the cause of lactic acidosis [16] when the therapeutic range is at <2μg/mL [17].

Guidelines with regard to the perioperative care of the National Institute for Health and Care Excellence (NICE), the French Society of Anaesthesia and Intensive Care Medicine (SFAR) and the British National Formulary recommend the discontinuation of metformin before surgery [5–7]. To this day no guidelines have been provided on its perioperative use issued by any international association of anesthesia. In our institution, metformin is not discontinued until the day of the surgery.

In our study we compared lactate levels and lactate clearance between patients treated with or without metformin undergoing cardiac surgery with the use of cardiopulmonary bypass (CPB). In this patient population metformin use was not associated with increased postoperative lactate levels or impaired lactate clearance. Oddly, metformin users showed lower lactate levels and a better clearance than the rest of the cohort at 12h postoperatively, as well as lower lactate concentrations at 24h postoperatively. This could be partially explained by a shorter mean CPB time of 10 minutes in metformin users, although such a time period is clinically rather short to account for a difference in lactate concentrations.

We also performed a subgroup analysis focusing on patients with diabetes mellitus. No differences were found in postoperative lactate concentrations and clearance between patients treated with metformin and diabetics treated with different antiglycemic agents.

These results are in accord with two other recently published studies. Nazer et al. [18] in 2017 and Bano et al. [19] in 2019 studying diabetic patients undergoing CABG reported no impact of metformin use up-until the night before surgery in postoperative lactate concentrations. Our study included not only CABG patients, but also patients receiving all types of cardiac surgery with the use of cardiopulmonary bypass (CPB). In addition to their action, in our population we also explored lactate kinetics. Compared to absolute lactate values, lactate clearance seems to be a more meaningful prognostic parameter. Rapid clearance of lactate is a strong indicator of a better outcome in numerous different critical care settings [10, 11]. Patients using metformin in our study had a better lactate clearance at 12h postoperatively, although a group effect was not demonstrated overall between the two groups.

Similar results were reported in different ICU patient settings. Doenyas-Barak et al. [20], studying diabetic patients in septic shock with lactic acidosis, also found better outcome results in metformin users. Moreover, Park et al. [2], studying patients with severe sepsis or septic shock, found that metformin users had higher lactate levels in the early phase of resuscitation which normalized over the initial 24-h period. Lee et al. [3], however, found no association of metformin with hyperlactatemia.

Timing of hyperlactatemia onset also plays an important role in prognosis. Early-onset hyperlactatemia defined chronically from the beginning of CPB to arrival in the ICU has been associated with an increased lactate production from the myocardium as well as from the peripheral tissues. This type seems to be a more severe form as compared to the late onset hyperlactatemia, occurring 6 to 12 hours post admission in the ICU, with the associated postoperative mortality of 14.9% and 3.6% respectively [21–23]. In our study, lactate concentrations decreased over time during the first 24 postoperative hours, and lactate clearance increased steadily in the same period. This pattern was the same in all patient groups studied implying no effect of metformin on the type of hyperlactatemia.

In order to explain further our results, it is vital to investigate the possible causes and types of hyperlactatemia post cardiac surgery in more detail. In this setting, different mechanisms were proposed to contribute to the final measured lactate concentration: liver dysfunction due to transient hypoperfusion, hypothermia, bacterial translocation and endotoxin release resulting from decreased splanchnic flow [24]. Moreover, the use of inotropes such as adrenaline also promotes lactate formation. We used the GFR MDRD formula to assess the renal function

A few of our patients in the elective procedures group exhibited postoperative lactic acidosis. In all those cases, postoperative low cardiac syndrome, cardiac tamponade, or extreme hypovolemia seemed to be the main causative factors for lactic acidosis.

There are several limitations to our study. First of all, this is a single centre retrospective study. Moreover, the number of patients included was small, and it is possible that including a larger number of patients would have shown a statistically significant difference between the study groups. However, in our results we did not detect even a tendency towards higher lactate levels in metformin users. On the contrary, a small, but significant, difference was noted in favor of the metformin users group. Finally, and most importantly, this is an observational study lacking a control group of metformin users in whom the drug was discontinued prior to the surgery and its results should be interpreted as such. Further studies including an adequately powered randomized controlled study are needed to define the proper timing of metformin discontinuation before the surgery.

Conclusions

In conclusion, in a post cardiac surgery ICU setting, using metformin up until the night before surgery was not associated with increased lactate levels or impaired lactate clearance. Further studies are necessary before issuing guidelines with regard to the proper preoperative use of metformin.

Authors' roles and individual contributions

Fotini Ampatzidou – conceptualization, methodology, validation, investigation, resources, writing – original draft.
Konstantinos Diplaris – methodology, software, formal analysis, writing – original draft.
Odysseas Drosos – formal analysis, investigation, writing - original draft.
George Drossos – Validation Writing – review and editing, visualization, supervision.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

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REVIEW PAPER

JMS Journal of Medical Science

Molecular characterization of multiple myeloma

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Keywords: multiple myeloma, KRAS, NRAS, mutations

Published: 2022-03-31

How to Cite: Kiremitci BZ, Gürler ES, Kiraz Y. Molecular characterization of multiple myeloma. Journal of Medical Science. 2022;91(2);e656. doi:10.20883/medical.e656

DOI: https://doi.org/10.20883/medical.e656

ABSTRACT

Multiple myeloma (MM) is a hematologic malignancy which occurs when plasma cells, a type of white blood cell, grow out of control and start to overproduce antibodies accumulating in the blood and bone marrow. Despite the recent advances, the survival rate for MM has not increased significantly which opens the need for identifying new molecular targets. This review article presents the most frequently observed gene mutations (KRAS (22.0%), NRAS (18.0%), DIS3 (9.3%), TTN (8.3%), ZNF717 (8.3%), TENT5C (7.3%), TP53 (7.3%) %), BRAF (6.3%), MUC16 (6.3%), RYR2 (5.4%), and LRP1B (5.4%)) in MM patients, with their rates, correlations, clinical significance, importance in the framework of MM, as well as potential novel targets collected from the literature. The genes and MM patients' dataset (211) were obtained from cBioportal. Summing up, in the study conducted in MM patients, 3 genes with the most frequent mutations were reported as KRAS, NRAS and DIS3. In addition, in the context of our literature reviews and the data obtained, it appears that the TZNF717, TTN, MUC16, RYR2 genes need further investigations within the framework of MM.

Introduction

Multiple myeloma (MM), or Kahler's Disease, is a disorder characterized by a malignant and uncontrolled division of antibody-secreting plasma cells (PCs) in the bone marrow. Even though the disease has a typical histologic diagnosis, it also presents a high level of genomic complexity, as well as significant differences in clinical features and patient survival [1]. Among all plasma cell neoplasms, MM is the second most common hematologic malignant disease with approximately 10% rate [2]. Due to its short survival time and high levels of fatal outcomes, it is frequently mislabeled as a rare disease. In fact, the proliferation of clonal PCs in the bone marrow is a defining feature of the disease and is marked by tumor cell secretion of monoclonal immunoglobulins (Igs) detectable in serum and/or urine. Accumulation of these abnormal PCs results in bone lesions and the destruction of bone tissue, bone marrow failure, and/or anemia [3].

Pathophysiology of MM comprises evolution in multiple stages that are monoclonal gammopathy of undetermined significance (MGUS), smoldering (asymptomatic) MM, symptomatic (intramedullary) MM, and extramedullary MM/plasma cell leukemia (PCL) [4]. The disease presents as the first step of monoclonal gammopathy of undetermined significance (MGUS). After the first step, the disease advanced to the subsequent ones, smoldering (asymptomatic) MM and symptomatic (intramedullary) MM. As the final stage of the disease, extramedullary or plasma cell leukemia is observed. MGUS evolves via branching evolution, as shown by many mutations and genomic aberrations at both the clonal and subclonal levels, leading to MM's genomic heterogeneity. The disease can be classified as hyperdiploid (having greater than the diploid number of chromosomes) or non-hyperdiploid (with fewer than the diploid number of chromosomes). In fact, hyperdiploid MM has usually been associated with a better prognosis [5].

Although there is still some ambiguity concerning the genomic nature of the disease, it has been established that the accumulation of abnormal PCs, chromosomal aberrations, hypermutated immunoglobulin genes, or dysregulation on MYC expression are significant causes in MM pathogenesis.

It was estimated that there would be about 34,920 new cases (1.8% of all cancer types) and about 12,410 deaths to occur due to MM in 2021. Additionally, the disease risk is higher for men than women [6]. According to the data obtained from different sources, over 50% of MM patients are reported to be over 60 ages, and nearly 3% of the patients are aged 40 and younger [7]. However, due to the developments in the understanding with regard to the genetic background of hematological neoplasm of PCs, the median survival time for patients has increased by three to four times in the last four decades [8].

The objective of this article is to analyze the genetic characterization of MM by using known genomic data and current knowledge in the literature. In view of such information, we aim to propose novel targets which could play a role in the disease diagnosis or/and prognosis, as well as in the development of new treatment methods.

Gene mutations in multiple myeloma

Samples from 211 patients using the cBioPortal for Cancer Genomics database [10] has allowed for the identification of gene mutations for MM. Parallel sequencing of paired tumor/normal sample of 203 MM patients has been performed [9–11]. According to the data obtained, significantly mutated genes have been listed. KRAS, NRAS, DIS3, ZNF717, TTN, TENT5C, TP53, BRAF, MUC16, RYR2, and LRP1B genes emerged as the eleven genes with the most frequent mutations. Results based on whole exome or whole genome sequencing of 203 MM matched tumor/normal sample pairs were retrieved from the cBioportal [10, 11]. As it is presented in the **Table 1**, some mutations of the genes are overlapping and seen in more than one sample. Even though the number of samples was greater for some mutations, the most common mutations are the following: KRAS (22.0%), NRAS (18.0%), DIS3 (9.3%), TTN (8.3%), ZNF717 (8.3%), TENT5C (7.3%), TP53 (7.3%), BRAF (6.3%), MUC16 (6.3%), RYR2 (5.4%), LRP1B (5.4%) (Figure 1A) which are mostly defined as missense mutations (putative driver and unknown significance). Gene altered in 67.32% of 205 cases, which equals 138 cases (**Figure 1A**).

Table 1. The analysis testing gene pairs across on OncoPrint. Gene mutations in pairs and separately in the study of MM patients

A	B	Neither	A Not B	B Not A	Both	Log2 Odds Ratio	p-Value	q-Value	Tendency
KRAS	DIS3	148	38	12	7	1.184	0.092	0.64	Co-occurrence
NRAS	ZNF717	156	32	12	5	1.022	0.17	0.64	Co-occurrence
KRAS	TENT5C	150	40	10	5	0.907	0.211	0.64	Co-occurrence
KRAS	RYR2	153	41	7	4	1.092	0.202	0.64	Co-occurrence
NRAS	TTN	155	33	13	4	0.531	0.368	0.64	Co-occurrence
NRAS	DIS3	153	33	15	4	0.306	0.46	0.64	Co-occurrence
KRAS	ZNF717	147	41	13	4	0.142	0.537	0.656	Co-occurrence

A

Mutated Genes (205 profiled samples)			
Gene	# Mut	#	Freq
KRAS	47	45	22.0%
NRAS	37	37	18.0%
DIS3	19	19	9.3%
ZNF717	18	17	8.3%
TTN	17	16	7.8%
TENT5C	15	15	7.3%
TP53	16	15	7.3%
BRAF	13	13	6.3%
MUC16	15	13	6.3%
RYR2	12	11	5.4%
LRP1B	13	11	5.4%

B

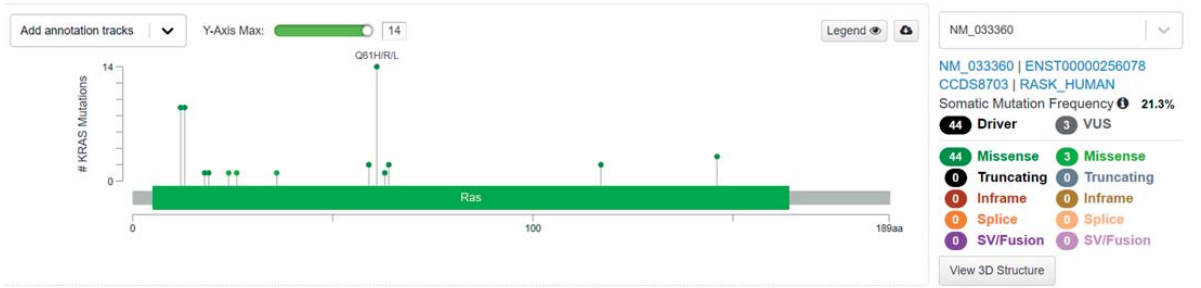
Figure 1. The most frequently mutated genes for 205 profiled samples from cBioPortal (*cBioPortal for Cancer Genomics*, n.d.-a). Figure 1A shows the mutated gene percentages. Figure 1B shows the genetic alterations of mutated genes for samples

KRAS mutations (mostly putative driver missense mutations) are seen in 22% of the samples whereas NRAS mutations (mostly putative driver missense mutations) are seen in 18% of the samples.

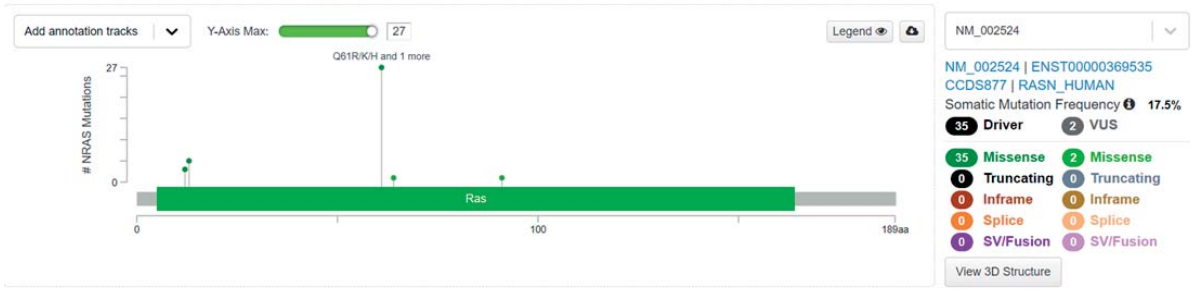
Although DIS3 mutations are found in 9%, ZNF717 mutation in 8%, and TTN mutation in 8% of the patients, they mostly contain unknown significance missense mutation (**Figure 1B**). Additionally, the locations of gene mutations are presented in **Figure 1B**. As seen in **Figure 1B**, there have been mutational overlaps. These are shown in **Table 1**, from the highest to the lowest numbers of co-occurrence mutations, according to the genes they overlap.

Figure 2 presents mutation types and locations of the KRAS, NRAS, DIS3 and ZNF717 genes with the highest mutation rates in MM. According to these data, KRAS mutations are missense mutations (47) and most of them are driver (44) mutations (**Figure 2A**). Similarly, the type which generates NRAS mutations also is a missense mutation (37) and most of them are defined as drivers (35) (**Figure 2B**). DIS3 mutation information consists of missense mutations and 7 of them are driver mutations (**Figure 2C**). Therefore, KRAS, NRAS and DIS3 genes can be considered as potential targets to inhibit the growth of cancer-causing cells. After emphasizing the importance of the mutations percentage in the abovementioned genes in MM patients, further studies should be conducted to address them. ZNF717 gene has been the most mutated gene, after KRAS, NRAS and DIS3 genes. In terms of the ZNF717 mutation, driver mutations are not defined, and there are missense mutations that have been indicated (17) (**Figure 2D**).

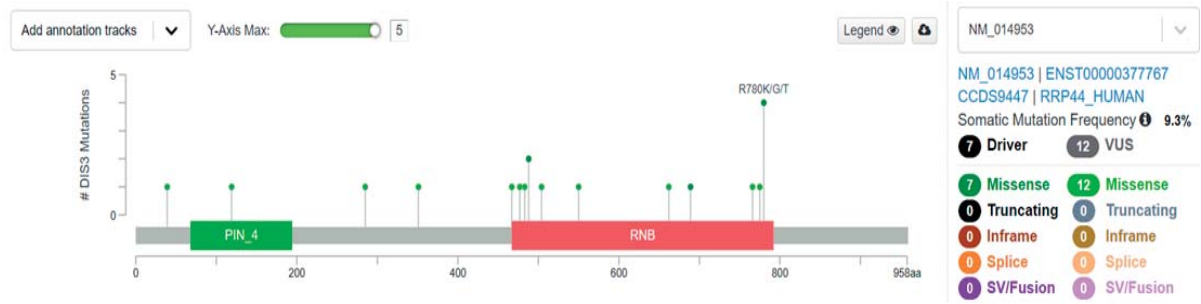
A. KRAS



B. NRAS



C. DIS3



D. ZNF717

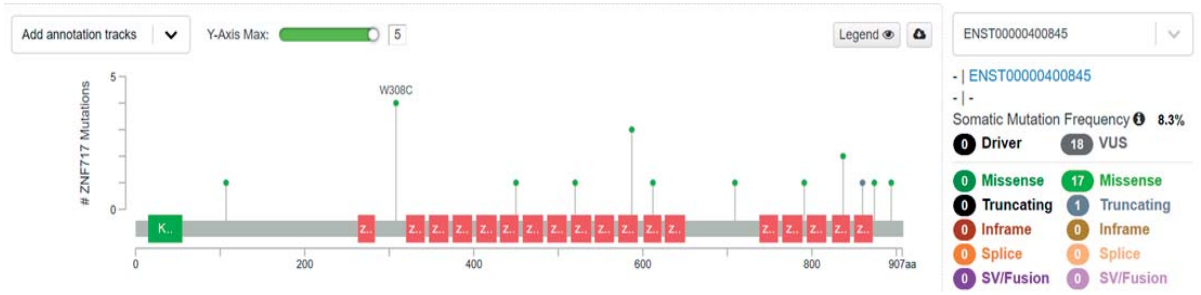


Figure 2. Representation of the most common mutations found on the most frequently mutated genes in MM dataset. A — KRAS, B – NRAS, C – DIS3, D — ZNF717 genes (*cBioPortal for Cancer Genomics*, n.d.-a)

Correlation Analysis between the Mutated Genes

As shown in **Figure 3** which has been obtained from the STRING database [12], KRAS, BRAF, and NRAS are the most related genes to each other from the curated databases. Even though the disease is seen as not fully curable, new advances in the treatment have shown that at least a small percentage of the patients may achieve the so-called operational cure [13]. Furthermore, Pasca et al. (2019) hypothesize that mutations in the KRAS/NRAS/BRAF genes are linked to a larger number of mutations per patient, and these genes are on the MAPK pathway checkpoint and/or MAPK inhibitors could be used as therapeutic

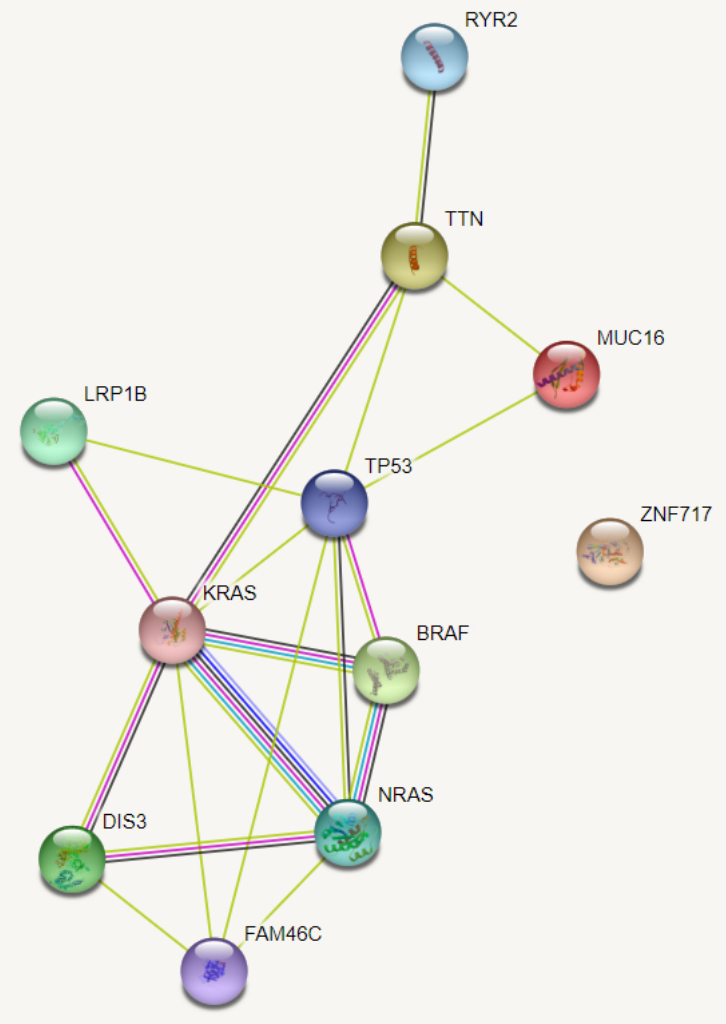


Figure 3. Physical network connection of the selected 11 genes from the STRING database. The purple and light blue edges show experimentally determined interactions. As a predicted interaction, black edges show the co-expression of genes (*11 Items (human) – the STRING Interaction Network*, n.d.)

agents for MM patients [14]. In addition, Kortüm et al. (2015) worked on fluorescence in-situ hybridization (FISH) and gene expression profiling to define the landscape of MM [15]. According to the mutation prevalence of the newly diagnosed patients; BRAF, NRAS, KRAS, TP53, DIS3, LRP1B are the genes that have multiple mutations and FAM46C has no mutation. Although, according to the cBioportal database, ZNF717 has an 8.3% mutation frequency [11], and there is text mining evidence for every 10 genes shown using yellow lines, ZNF717 has no physical network interaction with other mutated genes. Additionally, there is no protein homology evidence for the interested genes, although when the cluster tool of the database is used, BRAF, NRAS and KRAS genes are always clustered in the same group. As a physical network, an interaction is observed between only BRAF, NRAS, DIS3 genes which are referred to as 'driver' genes of MM from the curated and experimentally determined databases [16]. In addition, BRAF, NRAS, KRAS, and TP53 may be proto-oncogenes for a number of cancer types, such as thyroid, colorectal, or prostate cancer and have a role in the metabolic and signaling pathways [12].

CBio Gene Query Analysis

Data on which two genes were found together or separately in the study conducted with MM patients were obtained from cBioportal [11] (**Table 1**). This table contains information regarding gene

pairs in patients where mutations were found as co-occurring, separately or not observed at all. It is possible to access all possible gene pairs of interest from the cBioportal site [11]. In total, 7 patients with KRAS and DIS3 gene mutations were found, and these are the most common co-occurring gene mutations. Only KRAS mutation has been observed in 38 patients (without DIS3 mutation) and DIS3 mutation only in 12 individuals (without KRAS mutation). The number of individuals with the co-occurrence of the NRAS and ZNF717 genes has been recorded as 5. Moreover, 32 patients with only NRAS mutations (without ZNF717 mutations) and 12 patients with only ZNF717 mutations (without NRAS mutation) have been found. Likewise, mutations of KRAS and TENT5C have been observed in 5 patients. Those with only KRAS mutation and no TENT5C mutation have been recorded in 40 cases, and those with TENT5C mutation and no KRAS mutation have been recorded in 10 cases (**Table 1**).

Prognostic relevance of gene expressions

(See **Table 2**).

Significance of the identified gene signatures for MM

MM mutations have been identified by sequencing studies. KRAS, NRAS, TP53, FAM46C, DIS3, and BRAF mutations have a high risk of recurrence, as well as may play essential roles in the pathogenesis, progression, and prognosis of MM. Targeted sequencing analysis found the most common mutated genes to be the following: KRAS (36%), NRAS (20%), TP53 (16%), DIS3 (16%), FAM46C (12%). The induction of high-quality remission, including full response, is frequently the first treatment for MM. Treatment success necessitates the targeting of a diverse set of targets, including small subclones. In order to assess efficacy, it is essential to monitor gene changes in the tumor cell population under the pressure of developing treatments. Although mutation diversity constitutes an inherent feature of myeloma, it has been found that different genes in the same pathway cause multiple mutations (KRAS, NRAS, BRAF) in the same patient. Moreover, FAM46C and DIS3 have been found to be possible driver genes in MM. The finding of such driver gene mutations in MM has generated a considerable amount of expectations regarding the area of personalized treatment [20].

Table 2. Summary of gene and clinical significance. The summary of information regarding gene families, functions and clinical importance of KRAS, NRAS, DIS3, ZNF717, TTN, TENT5C, TP53, BRAF, MUC16, RYR2, and LRP1B genes, the most frequently mutations observed in MM patients

KRAS	The KRAS gene, a Kirsten Ras oncogene homolog from the mammalian Ras gene family, is responsible for producing a small GTPase superfamily protein. An activating mutation is caused by a single amino acid alteration. Lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, and colorectal cancer have all been linked to the transforming protein that results. Due to alternative splicing, two isoforms with different C-terminal regions are produced. Downstream signaling mechanisms of B Cell Receptor (BCR) and VEGF Signaling Pathway are two linked pathways. GTP binding is one of the Gene Ontology (GO) annotations for this gene, whereas HRAS is an essential paralog of this gene.
NRAS	This is an N-Ras oncogene that codes for a membrane protein which transports information between the Golgi apparatus and the plasma membrane. The ZDHHC9-GOLGA7 complex regulates this shuttling through palmitoylation and depalmitoylation. A guanine nucleotide-exchange factor activates the encoded protein, which possesses intrinsic GTPase activity, while a GTPase activating protein inactivates it. Diseases, such as somatic rectal cancer, follicular thyroid cancer, autoimmune lymphoproliferative syndrome, Noonan syndrome, and juvenile myelomonocytic leukemia, have been associated with NRAS mutation. Downstream signaling mechanisms of B Cell Receptor (BCR) and VEGF Signaling Pathway are the two linked pathways. GTP binding is one of the Gene Ontology (GO) annotations for this gene. HRAS is an essential paralog of this gene.
DIS3	The protein-coding gene DIS3 (DIS3 Homolog, Exosome Endoribonuclease, And 3'-5' Exoribonuclease) is found in exosomes. Plasma Cell Neoplasm and Perlman Syndrome are two diseases associated with DIS3. Unfolded Protein Response (UPR) and Gene Expression are the two pathways that are connected to it. RNA binding and endonuclease activity are two Gene Ontology (GO) annotations for this gene. DIS3L is a significant paralog of this gene.
ZNF717	Kruppel-associated box (KRAB) zinc-finger protein, which relates to a wide set of transcriptional regulators in mammals, is encoded by ZNF717. These proteins bind nucleic acids and are involved in a variety of physiological activities, such as cell proliferation, differentiation, and apoptosis, as well as viral replication and transcription regulation. On chromosome 1, a pseudogene of this gene was discovered. Gene Expression is one of its associated pathways. Nucleic acid-binding and DNA-binding transcription factor activity are two GO annotations connected to this gene. ZNF268 is a significant paralog of this gene.

Table 2 continued.

TTN	This gene produces a substantial amount of striated muscle protein. The product of this gene is split into two parts: an N-terminal I-band and a C-terminal A-band. On either side of a PEVK region rich in proline, glutamate, valine, and lysine, the I-band, which is the molecule's elastic component, comprises two areas of tandem immunoglobulin domains. The A-band comprises a combination of immunoglobulin and fibronectin repeats, as well as kinase activity, and is hypothesized to operate as a protein-ruler. A single titin molecule spans half the length of a sarcomere thanks to an N-terminal Z-disc area and a C-terminal M-line region which bind to the Z-line and M-line of the sarcomere, respectively. Titin also functions as an adhesion template for the formation of contractile machinery in muscle cells, since it comprises binding sites for muscle-associated proteins. It has been discovered as a chromosomal structural protein. In the I-band, M-line, and Z-disc sections of titin, there is a lot of variation. Variability in the I-band area contributes to variances in titin isoform elasticity and, as a result, to differences in elasticity of various muscle types. Autoantibodies to titin are generated in patients with the autoimmune diseases, such as scleroderma, and mutations in this gene are associated with the familial hypertrophic cardiomyopathy 9. Striated Muscle Contraction and Response to Elevated Platelet Cytosolic Ca2+ are two linked mechanisms. Nucleic acid binding and the same protein binding are two Gene Ontology (GO) annotations for this gene.
TENT5C	TENT5C is a Protein Coding gene (Terminal Nucleotidyltransferase 5C). Smoldering Myeloma and Monoclonal Gammopathy Of Uncertain Significance are two diseases linked to TENT5C. TENT5A is a significant paralog of this gene. Nucleotidyltransferase is a non-canonical poly(A) RNA polymerase that improves the stability and expression of mRNA. It primarily targets mRNAs that encode endoplasmic reticulum-targeted proteins and may be involved in the induction of cell death.
TP53	The transcriptional activation, DNA binding, and oligomerization domains of TP53 encode a tumor suppressor protein. The encoded protein reacts to a variety of cellular stressors by regulating target gene expression, resulting in cell cycle arrest, apoptosis, senescence, DNA repair, or metabolic alterations. TP53 mutations have been linked to several human malignancies, including hereditary tumors, such as Li-Fraumeni syndrome and Osteogenic Sarcoma. Multiple transcript variants and isoforms occur from alternative splicing of this gene and the utilization of various promoters. The usage of the various translation start codons from identical transcript variants has also been proven to result in additional isoforms. PI3K/AKT activation and Cell Cycle Mitotic are two linked pathways. DNA-binding transcription factor activity and protein heterodimerization activity are two Gene Ontology (GO) annotations for this gene. TP73 is a significant paralog of this gene.
BRAF	The BRAF protein is a member of the RAF family of serine/threonine protein kinases. This protein influences cell division, differentiation, and secretion via modulating the MAP kinase/ERK signaling pathway. Mutations in this gene, most notably the V600E mutant, are the most often detected cancer-causing mutations in melanoma, although they have also been found in non-Hodgkin lymphoma, colorectal cancer, thyroid carcinoma, non-small cell lung carcinoma, hairy cell leukemia, and lung adenocarcinoma. BRAF mutations are also linked to cardiofaciocutaneous, Noonan, and Costello syndromes, all of which have similar characteristics. Development Slit-Robo Signaling and CNTF Signaling are two pathways that are associated with it. Calcium ion binding and transferase activity, which transfers phosphorus-containing groups, are two Gene Ontology (GO) annotations for this gene. RAF1 is a significant paralog of this gene.
MUC16	The protein encoded by this gene belongs to the mucin family. Mucins are large molecular weight, O-glycosylated proteins located on the apical surfaces of epithelial and serve a vital role in establishing a protective mucous barrier. This protein is hypothesized to contribute to the formation of a barrier which protects epithelial cells from pathogens. This products of this gene have been used as markers for several malignancies, with higher expression levels linked to poorer outcomes. Clear Cell Adenocarcinoma and Ovarian Cyst are two diseases linked to MUC16. Defective C1GALT1C1 causes Tn polyagglutination syndrome (TNPS) and Diseases of Glycosylation are two of its linked pathways.
RYR2	This gene encodes a ryanodine receptor present in the sarcoplasmic reticulum of the heart muscle. The encoded protein is part of the calcium channel delivering calcium to the heart muscle and is made up of a tetramer of ryanodine receptor proteins and a tetramer of FK506 binding protein 1B proteins. Stress-induced polymorphic ventricular tachycardia and arrhythmogenic right ventricular dysplasia are linked to mutations in this gene. Activation of cAMP-Dependent PKA and CREB Pathway are two similar pathways. Calcium ion binding and protein kinase binding are two Gene Ontology (GO) annotations for this gene. RYR3 is a significant paralog of this gene.
LRP1B	A member of the low-density lipoprotein (LDL) receptor family is encoded by LRP1B. Due to their interactions with a range of ligands, these receptors perform a variety of functions in a proper cell function and development. This gene is disrupted in a variety of cancers. Lung cancer and Meier-Gorlin Syndrome 2 are two diseases associated with LRP1B. Calcium ion binding and low-density lipoprotein particle receptor activity are two Gene Ontology (GO) annotations for this gene. LRP1 is an essential paralog of this gene.
Ref	[17–19] Sources: https://www.genecards.org/ https://civicdb.org/home https://www.ncbi.nlm.nih.gov/

Copy-number changes and translocation studies performed on the newly diagnosed myeloma samples are examined in data sets with a long-term follow-up, and the effects of mutations are discussed. Although the most frequently mutated genes in the study were NRAS, KRAS, and BRAF, they constituted 44% of the cases. BRAF and DIS3 mutations showed an effect accompanying classical risk factors. 44% of patients with hypoactive/kinase-dead BRAF mutations presented concomitant changes in KRAS, NRAS, or activating BRAF mutations. They may have a role in the oncogenesis of MM by means of facilitating MAPK activation, which in turn may contribute to chemotherapy resistance. Thus, the findings show how important mutation screening is for better understanding newly diagnosed MM and may lead to patient-specific mutation-driven therapy methods [21].

Clonal evolution drives tumor progression, chemotherapy resistance, and relapse in cancer are known mechanisms; however, clonal selection induced by therapeutic pressure in MM has not been investigated to such a great extent. To investigate this problem, researchers used large-scale targeted sequencing of bone marrow PCs in MM patients at the diagnosis and recurrence following the same intense therapy. The most common mutations found at the diagnosis were KRAS (35%), NRAS (28%), DIS3 (16%), BRAF (12%) and LRP1B (12%). The mutational burden remained unaltered at recurrence. Chemotherapy resistance and recurrence may be caused by the newly acquired mutations in myeloma drivers, as well as (sub)clonal mutations which were present prior to the therapy [22].

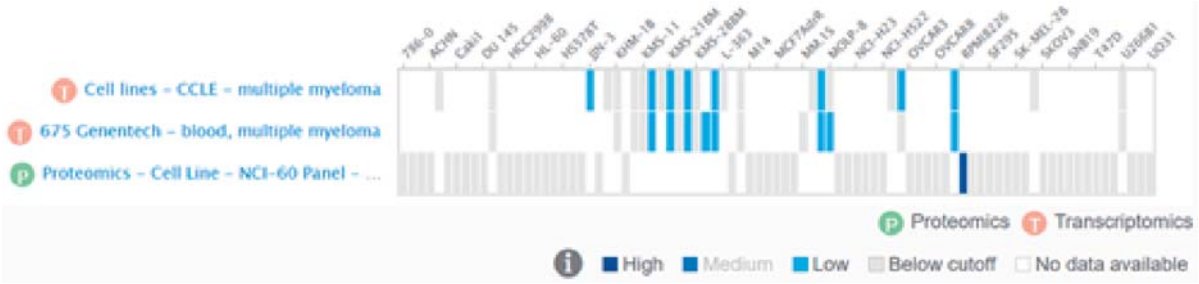
A Genome-wide association (GWAS) study found the 2q22 (rs61070260) variation affecting MM risk, and subsequently the association between rs61070260 in LRP1B and MM was evaluated. The results demonstrated that variation in LRP1B was highly correlated with MM susceptibility. In addition, a linkage disequilibrium (LD) study of this variation indicated an LD block containing exons 26-27-28 of the LRP1B gene, and the following sequencing analysis found three SNPs in exons 26 and 28 of LRP1B (rs762074421, rs756168629, rs113600691). A missense mutation leading in a transition from arginine to histidine at position 1661 of the LRP1B protein was not detected for the SNP rs756168629 in exon 26, and this mutation was estimated to be deleterious by SIFT and PolyPhen. These findings solidify the notion that LRP1B is a disease-associated gene involved in the development and progression of MM [23].

Although FAM46C is one of the most recurrently mutated genes in MM, its role in the pathogenesis of the disease has not yet been determined. According to one of the studies, wild-type (WT) FAM46C overexpression has induced cytotoxicity in MM cells. FAM46C mutations observed in MM patients eliminated this cytotoxicity, giving the mutant phenotype a survival benefit. In fact, FAM46C mutation is implicated in myeloma cell growth and survival, and this gene mutation is identified as a contributor to myeloma pathogenesis and disease development by means of the disruption of plasma cell differentiation [24].

Although the FAM46C gene is frequently mutated in MM, it has been found to encode a non-canonical poly(a) polymerase nc(PAP). Nevertheless, its target mRNAs and its role in the disease pathogenesis are much less known. A recent study using CRISPR-Cas9 technology and gene expression analysis has found that inactivation of FAM46C in MM down-regulated several mRNAs encoding Igs and ER-resident proteins. Surprisingly, poly(A) tail length determination tests have indicated that FAM46C expression is induced throughout plasma cell (PC) differentiation and that Ig mRNA encoding chains are substrates of ncPAP. In contrast, FAM46C loss increases the potential to migrate via upregulating the metastasis-associated lncRNA MALAT. The research has found that Ig mRNAs are targets of FAM46C, although they also revealed that this protein has a significant function in increasing antibody production during PC maturation, suggesting that its activity as a tumor suppressor is linked to the inhibition of myeloma cell migration [25].

Another factor associated with poor outcomes in MM is alterations of the tumor suppressor TP53. Once the oncogenic stress or DNA damage activates the p53 gene, it causes cell cycle arrest, or apoptosis depending on the biological environment, and its inactivation is linked to drug resistance in MM. The frequency of TP53 mutations increases with the development of the disease, from 5% at the diagnosis to 75% in late relapses [26].

A.



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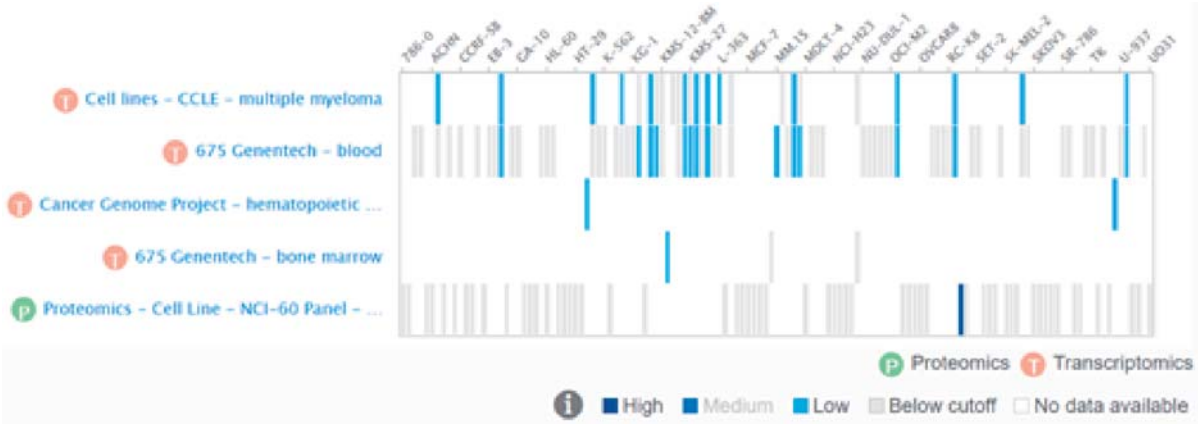

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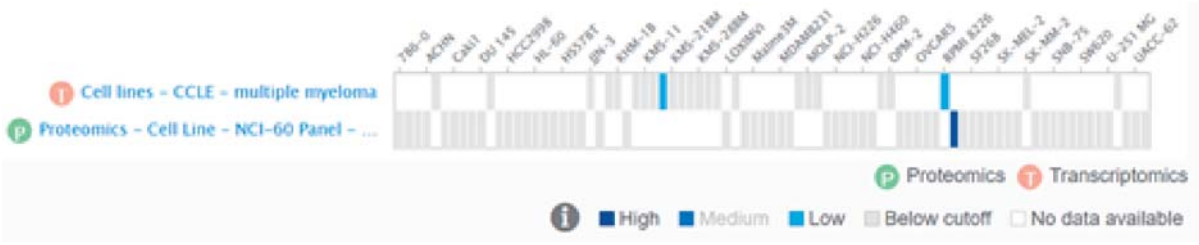

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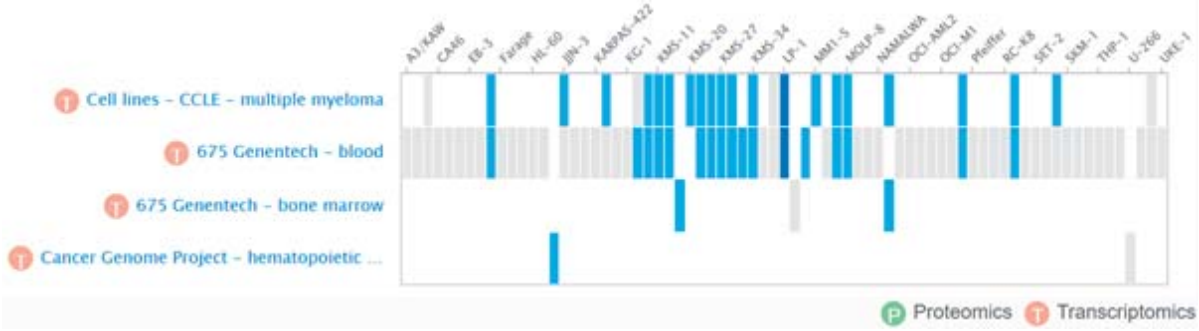

Figure 4. Figure 4. The expression pattern of RYR2, TTN, MUC16 and ZNF 717 genes in MM. A – RYR2, B – TTN, C – MUC16, D – ZNF717. 3 different experiments of RYR2 gene for MM. Two of them are transcriptomics experiments with CCLE – MM and 675 Genentech blood, MM, and the third is Proteomics – Cell Line – NCI 60 Panel – not applicable (Figure 4A). 5 different experiments have been performed showing the expression of TTN gene in MM (CCLE – MM, 675 Genentech – blood, Cancer Genome Project – Hematopoietic system – MM, 675 Genentech – Bone Marrow and Proteomics – Cell Line – NCI 60 Panel – not applicable) (Figure 4B). 2 experiments showing MM expressions of MUC16 gene were recorded (CCLE – MM and Proteomics – Cell Line – NCI 60 Panel – not applicable) (Figure 4C). Transcriptomics experiments have been performed in 4 different experiments (CCLE – MM, 675 Genentech – Blood, 675 Genentech – Bone Marrow, Cancer Genome Project – Hematopoietic system – MM) showing the expressions of the ZNF717 gene in MM (Figure 4D)

The expression levels of the RYR2, TTN, MUC16 and ZNF717 genes in MM homo sapiens are shown with the data obtained from the Expression Atlas (https://www.ebi.ac.uk/gxa/home) [27] (**Figure 4**). Expressions of these genes in cell lines were examined and tested. Generally, the expression levels of these genes were observed to be low (**Figure 4**). In fact, the low expression levels of these genes and their relationship with patient samples should be investigated at the molecular level. With these research results, a new approach can be obtained for the treatment of MM. Therefore, further studies are needed to clarify the mechanism and possible role of these genes in MM.

According to the graph shown in **Figure 5** obtained from cBioportal [11], the patients' cause of death (205 samples) changes depending on the mutated genes. Disease progression has been the cause of death for approximately 30% of the KRAS gene mutated patients, ~10% of DIS3, TTN, TENT5C or TP53 genes mutated patients, ~5% ZNF717 or LRP1B genes mutated patients. Additionally, disease progression has also been established to be the cause of death in the case of 20% of NRAS gene mutation patients. Although NRAS is the second most frequently mutated gene in MM, samples with NRAS gene mutations have a higher rate of cause of death defined as 'other' on the database (~ 40%). Moreover, a higher proportion of causes of death described as 'other' are found in patients with mutations on DIS3, TP53, BRAF, MUC16 and LRP1B. On the other hand, the frequency of RYR2 mutation ranks as 10th in Figure 1b, yet there is no available information regarding the cause of death of patients. According to the graph, death due to disease progression has the highest percentage only in patients with KRAS mutations. [11].

Novel targets (novel prediction markers)

Titin (TTN) is the largest known protein and the third most abundant filament in the cardiac and skeletal muscle, performing developmental, mechanical, and regulatory roles. With the widespread use of NGS, TTN has started to be reported as one of the main genes emerging in human hereditary diseases. Due to the essential role of TTN's, its size, modular structure, and numerous protein interactions, individual mutations may present greatly varied biological consequences and clinical symptoms. In the majority of cases, TTN mutations have been linked to a predominantly cardiac phenotype [28]. Thus, the impact on MM is of interest, given the potential for a diversity of its biological consequences.

According to the reports, TTN mutations may play an essential role in the immunotherapy of solid tumors and gastric cancer. However, no relationship has been shown between the TTN mutation and MM immunotherapy. Although research regarding the association between TTN mutation status and prognosis remains unclear, it is possible it may offer a new perspective to disease treatment [29].

Technical challenges and budgetary constraints make it difficult to achieve full Sanger Sequencing of TTN consisting of 364 exons. In addition, target enrichment strategies and sin-

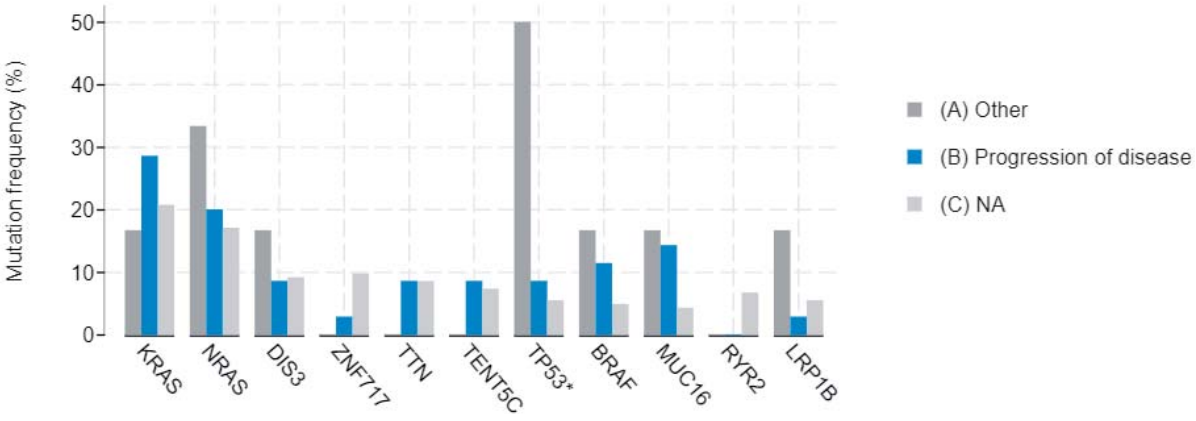

Figure 5. Patients' cause of death plot in correlation with mutation frequencies (*cBioPortal for Cancer Genomics*, n.d.-a)

gle-molecule sequencing studies used with second-generation sequencing may allow for diagnostic assays in this gene [28].

Recent NGS studies have shed light on cancer and its heterogeneity using bulk tumor samples. Tumor initiation and evolution are driven by sequential genetic changes in single cells, and single cell-sequencing has the potential of adding new insights into cancer studies in the bulk tumor genomic data [30]. In a study involving the use of these improvements, the clonal evolution of HBV-associated hepatocellular carcinoma has been approached by single-cell genome sequencing. It is widely recognized that the protein encoded by ZNF717 belongs to the zinc finger family, which plays an important role in the regulation of gene expression. Nevertheless, the precise role of ZNF717 in tumor pathogenesis has not been fully understood yet. In addition, although the effect of ZNF717 on MM remains unknown, its effect on hepatocellular carcinoma (HCC) has been investigated and the results have been shared in the past years. These results suggest that the tumor inhibitory effect of ZNF717 in HCC is likely mediated through the regulation of the IL-6/STAT3 pathway. Therefore, it can be assumed that ZNF717 is known to act as a tumor suppressor in HCC [30].

Furthermore, according to the studies from the STRING database, it has been observed that some genes, such as RAF1, UBE3A, BARD1, EXOS5C, AURKA, PIK3CA, RPA1, RPA3, SKIV2L2, are co-expressed with the BRAF, KRAS, and NRAS genes which, in turn, are the most frequently mutated and most related genes with MM. The conducted studies have demonstrated that microRNA-497 (miR-497) has a role as a tumor suppressor for several cancer types, and it has been proven that MiR497 overexpression inhibited MM cell proliferation, while promoting apoptosis by decreasing Raf-1 expression [31]. Additionally, SIRT1 is activated by a proteasome inhibitor, and it deacetylates GLI2 to improve hedgehog signaling (Hh) and treatment resistance in MM [32]. MDM2 gene is another proliferative gene for MM, since the overexpression of MDM2 enhances the survival and proliferation of MM cells.

Even though there co-expression evidence on the STRING database for UBE3A and BARD1 genes, these genes are only investigated in some cancers, such as cervical cancer (UBE3A) and leukemia (related with BARD1); however, there are no studies demonstrating their association with MM.

Methodology

The studies "Broad, Cancer Cell 2014" were selected from the cBioPortal database [10,11] and searched for "query by gene". In total, there were 205 samples containing mutation data out of 213 samples. Subsequently, the most frequent eleven genes (KRAS, NRAS, DIS3, TTN, ZNF717, TENT5C, TP53, BRAF, MUC16, RYR2, LRP1B) from 205 profiled samples were chosen for further research. Therefore, in order to understand the connection between the abovementioned eleven genes, a physical network analysis was conducted by the STRING database. Using the text-mining evidence data of the STRING database, literature research about the studied genes was completed. In cBioPortal Query Analysis [10, 11], mutation profiles (205 samples) of the chosen genes in 211 samples were found by the OncoPrint tool. Next, to understand the tendency of the genes, the Mutual Exclusivity tool was used and Table 1 was generated. 55 pairs of the genes were analyzed on the database and seven of the most paired genes were shown in Table 1. 'Mutations' tool of cBioPortal was used to understand mutation types and somatic mutation frequencies [11] (from Figure 4 to Figure 14). In the literature review, summary for each gene and clinical significance information was accessed through PubMed. Table 2, which contains the summary for all the investigated genes and their importance was created from the GeneCards, CivicDB and NCBI databases [17–19] Subsequently, the importance of the genes in MM was researched by means of literature survey. Then, expression results of the eleven genes were identified on the basis of the Expression Atlas database [27]. To analyze the patients' cause of death (disease progression / other causes) 'Plots' tool of cBioPortal [10, 11] was used and the 'cause of death table' was generated. In the final step of the analysis, to find and decide on the novel targets for the disease, the STRING database was used and the co-expressed genes were identified as the new targets.

In total, data comprising 211 MM samples was used from the cBioportal. These samples included 205 cases of patients where mutation data were found. Additionally, according to Lohr et al 203 samples had tumor-normal pairs which could be further analyzed [9]. Therefore, although the total number of 205 patients was provided on the website, the actual number of samples with tumor-norm pairs was determined to be 203. Furthermore, when selecting the abovementioned 11 genes for further analysis, it was observed that the number of samples should be 138. In fact, of the 203 samples studied, 138 had mutations in the 11 genes that were investigated and which were thought to be significant for MM (separately or together).

Conclusions

According to the study involving MM patients from the cBioPortal data set, ZNF717 mutation has been seen in 17 patients, TTN mutation in 17 patients, MUC16 mutation in 13 patients, and RYR2 mutation in 11 patients. Since the effect and mechanism of these mutations on MM have not been studied yet, more studies are necessary to investigate the roles of these genes in MM progression. Moreover, according to the STRING database studies, BRAF, NRAS, and KRAS have been found to be the most related genes with one another (co-expression, database, and experimental evidence) which affect MM. Additionally, samples have been studied in both of these databases, and the frequency of gene mutations has been found to vary, with some genes, such as DIS3, or TTN, proven to affect the disease.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.
Funding sources
There are no sources of funding to declare.

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REVIEW PAPER

JMS Journal of Medical Science

Recent developments in the use of activated charcoal in medicine

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DOI: https://doi.org/10.20883/medical.e647

Keywords: activated charcoal, adsorption, surface area, toxins

Published: 2022-05-31

How to Cite: Hassen JH, Abdulkadir HK. Recent developments in the use of activated charcoal in medicine. Journal of Medical Science. 2022;91(2);e647. doi:10.20883/medical.e647

ABSTRACT

One of the raw forms of graphite is activated charcoal which has an extensive surface area allowing for the adsorption of a wide range of chemicals. It possesses the strongest physical adsorption forces of the available materials, as well as the largest volume of adsorbing porosity. Activated charcoal acts as an adsorbent, collecting and storing substances in the gastrointestinal tract, reducing or blocking absorption in the bloodstream. The ingested toxins interact with charcoal by recycling toxins in the intestinal cavity. In cases where the drug has not been absorbed from the abdominal system, it is recirculated through the liver and intestines or by means of passive diffusion or active secretion. The article aims to review the most recent advances in the use of the activated charcoal, including the dose, how charcoal acts in the body, the mechanism of action, administration, contraindications, as well as the impact of various factors on the adsorption process. In addition, we also discussed numerous medical applications of activated charcoal.

Introduction

Activated charcoal is a porous carbonaceous adsorbent in which the crystalline structure is damaged in the course of production, resulting in the formation of unstable and inhomogeneous pores in terms of energy and activity [1]. These pores are usually located on the outer surfaces of activated charcoal, although they can also be found inside. In terms of adsorption capacity, activated carbon has a larger capacity than any other adsorbent, and its surface area fluctuates from 300 m^2/g to 5000 m^2/g [2].

It is possible to distinguish two types of activated charcoal, i.e. powdered and granular. In comparison with carbons with larger particle sizes, powdered, micron-sized activated charcoal particles are milled from millimeter-sized granular activated charcoal, and demonstrate faster kinetics as well as a greater capacity for contaminants removal. The particle size of granular activated carbons generally ranges between 0.2 mm to 5 mm; moreover, it provides clean handling and tends to last longer than powder form [3]. Carbonization of the raw material is the first step in the production of activated charcoal, in which

thermal decomposition occurs in the absence of air. The majority of the hydrocarbons are first eliminated as gases during carbonization, without the use of chemical agents, in a process known as pyrolytic decomposition, also referred to as destructive distillation. In the course of the process, when coal is heated, chemical changes occur which result in the evaporation of gases and condensable vapors, thus, leaving a solid residue consisting almost entirely of carbon. The bituminous coal softens during this process, fuses and resolidifies to form coke, a porous substance rich in carbon. The carbonized products formed in this manner typically exhibit only a low adsorption capacity due to their underdeveloped pore structure and small surface area. This, in turn, improves in the process of activation, which converts the carbonized raw material into a form having the greatest number of scattered pores of various shapes and sizes, and producing a large and rich surface product [4–6]. The activation process can be performed chemically or physically. Chemical activation is the process of impregnating the precursor substance with dehydrating materials, such as NaOH, KOH, $ZnCl_2$, K_2CO_3, and H_3PO_4, to affect the pyrolytic decomposition of the precursor material, reduce the activation temperature, and prevent the production of tar. Physical activation includes partial gasification of the carbonaceous material at high temperatures in an inert ambient, followed by activation with oxidizing gases, such as CO_2, steam, air, or a mix of these [7, 8].

Since activated charcoal has been widely used, a number of sources have been involved in its preparation, which comprise nutshells [9–11], apricot stones [12], pistachio-nut shells [13, 14], agriculture waste [15, 16], almond shells [17], palm shell [18], wood apple fruit shell [19], sorghum waste [20], coir pith [21], olive stones [22], rubber tree [23], tea waste [24], and cotton stalk [25]. Furthermore, it has been also prepared from hair [26], bovine horns [27], waste tires [28], and several other sources. Several methods have been involved in the modification of activated charcoal to increase its surface area and enhance its adsorption properties. These methods include treating the material with different chemicals suitable for this purpose, e.g. citric acid [29], K_2CO_3 [30], $KMnO_4$ [31], $ZnCl_2$ [32], O_3 [33], H_2O_2 [34], H_3PO_4 [35], and NaOH [36]. The review aims to describe the recent medicinal uses and the benefits of activated charcoal application.

Dose of Activated Charcoal

Activated charcoal does not have any absolute recommendations for its use [37]. A single dose for adults is 10 times the weight of the toxic substance ingested, up to maximum of 100 grams. Numerous studies aiming to test the ability of activated charcoal to slow absorption with various charcoal-to-toxin ratios established a carbon-to-drug ratio of 10:1 [38]. Additionally, in order to test the efficacy of activated charcoal:drug ratios of 1:1, 2:1, 4:1, and 8:1 a rat model was involved for phenobarbital, chloroquine, and isoniazid exposures. At 1:1 ratios, absorption was reduced by 7%, 20%, and 1.2%, respectively, while at 8:1 ratios, absorption was reduced by 89%, 96%, and 80% [39]. Multiple dosages of activated charcoal have been effective in various circumstances, which may be helpful in the removal of a wide range of drugs [40–44]. The process includes two or more doses of activated charcoal in a row for the effective toxin removal. Administering multiple doses increases the excretion rate of a drug which has already been absorbed. In fact, this process removes drugs which are subject to significant entero-hepatic reabsorption, or exhibit a high affinity for charcoal and can be transported through the intestinal-capillary junction to be further absorbed. A study on the removal of fexofenadine by activated charcoal indicated that the amounts of the drug adsorbed following a repeated dose of activated charcoal increased by about 70% [45]. Aspirin, carbamazepine, dapsone, cyclosporine, dextropropoxyphene, digoxin, digitoxin, meprobamate, nadolol, nortriptyline, phenobarbitone, phenytoin, piroxicam, valproate, sotalol, and theophylline are all improved by the repeated treatment with oral activated charcoal. In severe poisonings, repeated dosing with oral activated charcoal appears to be necessary until the patient's condition have improved, or up to the point where plasma concentrations have dropped to the non-toxic levels. Activated charcoal doses of 20 to 50 g dissolved in water are usually administered every 4 to 6 hours for 1 to 2 days. Constipation is not a frequent adverse reaction, however, laxatives, such as sorbitol or lactulose, can be used in conjunction with

Table 1. Chemicals in which multiple doses of activated charcoal improve clearance

Chemicals names	
Amitriptyline	Nadolol
Carbamazepine	Phenobarbital
Cyclosporine	Phenylbutazone
Dapsone	Phenytoin
Dextropropoxyphene	Piroxicam
Digitoxin	Propoxyphene
Digoxin	Quinine
Disopyramide	Quinine Sotalol
Theophylline	

charcoal [38]. A 50–100 g initial dose followed by 30-50 g supportive doses every 2-6 hours is a suitable protocol [46]. Maintenance doses from charcoal in the intestines adsorb the toxin once it has been released back into the bowels, preventing a delayed peak in serum levels [47]. Some chemicals in which their clearance is increased by multiple doses are shown in **Table 1** [48].

Mechanism of Action

Activated charcoal acts as an adsorbent, which captures chemicals in the gastrointestinal (GI) system and subsequently retains them within the charcoal, thus, decreasing or preventing them from being absorbed into the bloodstream [37, 49, 50]. Orally given activated charcoal does not undergo absorption through the intestinal lumen and acts in its unaltered form within the gastrointestinal tract. If the drug has not been absorbed at this location, the ingested toxins interact with charcoal by recirculating the toxins into the intestinal lumen by either enterohepatic recirculation or entero-enteric recirculation through passive diffusion or active secretion [51]. The activated charcoal adsorptive surface comprises a variety of chemical forms, including carbonyl and hydroxyl groups that adsorb toxic chemicals with varying affinities [52]. Moreover, particle size, pore size, surface area, solubility, temperature, pH, the presence of inorganic ions, and other factors, have all been shown to alter its adsorptive activity in vitro [53]. Activated carbon adsorbs toxins in their non-ionized forms to a better extent, whereas adsorption is less common in polar, water-soluble compounds. Due to its pharmacodynamics, activated charcoal is most effective in adsorbing non-polar, poorly water-soluble organic toxins best [54].

Administration

When ingested poison is still present in the gastrointestinal tract, and when it is thought that the benefits of avoiding toxin absorption outweigh the dangers of providing activated charcoal, the patient should be administrated a charcoal dose. Activated charcoal can be provided orally, or by means of nasogastric and orogastric tubes [55]. Activated charcoal is available as carbon tablets, powder, or granulate. Powder or granular activated charcoal, where the doses ranging from 10 to 100 g, appears to be more feasible in clinical practice. In contrast, carbon tablets, only contain 250 mg of carbon, therefore, a significant number of tablets would be necessary to generate an acceptable carbon surplus. Any liquid can be used to suspend activated charcoal in, although water is the preferred medium [56].

Risks of Use

Activated charcoal has long been thought to be safe in the treatment of poisonings; however, this assumption, is currently being questioned [57]. Vomiting, corneal abrasions, and lung aspiration comprise the risks associated with single-dose activated charcoal delivery. Despite a few caveats to using activated charcoal, it still remains a good treatment option [58]. The most significant consequence following the activated charcoal administration is pulmonary aspiration [50]. Aspiration occurs when a nasogastric tube is accidentally inserted into the trachea instead of the stomach [59, 60]. Regardless of whether activated charcoal is present, gastric content aspiration may lead to severe airway obstruction, bronchospasm, hypoxemia, and pneumonia [61]. In addition, according to one of the studies, a charcoal-containing empyema developed in one patient [62]. It is vital to bear in mind that intubation for an extended period of time, death [50], and permanent lung injury [63] all constitute the possible negative outcomes. Activated charcoal should only be administered to cooperating patients, since. In terms of not fully conscious patients, the administration is not recommended, since the swallowing reflexes are impaired and there is a considerable risk of aspiration. In acute intoxication, intubation is required in order to secure the airways prior to

activated charcoal administration through a gastric tube [56]. Other contradictions involve the cerebral seizures risk and dysphagia [64].

Combination with Laxatives

Over the years, a common practice was a simultaneous administration of a laxative, such as sodium sulfate at the same time; nevertheless, this is no longer recommended. In certain situations, a laxative may be given following the ingestion of a medication reducing gastrointestinal movement activity. This is also a frequent method in the case of constipation, which may be followed with a dose of a bowel stimulant. If polyethylene glycol electrolyte solution and activated charcoal were given at the same time, the substances would bind together, limiting the adsorption capacity, therefore, they are administered individually (usually at two-hour intervals) [56, 65]. The sweetener and laxative agent, sorbitol, has been linked to emesis [37].

Effect of pH

The adsorption capacity of activated charcoal is affected by the pH of the environment. Drugs are most effectively adsorbed on activated charcoal in their undissociated form, whereas acids demonstrate greatest adsorption at low pH and bases at high pH [66]. In fact, it was found that the pH has a clear effect on the adsorption of aspirin [67], caffeine [68], paracetamol [69, 70], acetampirid pesticide [71], carbendazim and linuron [72], methylparaben [73], and many other compounds.

Effect of Time

The antidotal efficacy of oral activated charcoal is undoubtedly affected by delaying delivery after drug ingestion. As a result, it should be supplied as soon as possible [38]. The delay in administering activated charcoal and the amount given is the most critical determinants of its efficacy in acute poisonings. Activated charcoal should be given as a water suspension as soon as possible following toxic substance ingestion, preferably within 30 minutes [74].

Effect of Gastrointestinal Content

Similarly to any other competing solute, gastrointestinal contents are likely to affect drugs adsorption on activated charcoal. Although the presence of food in the stomach of individuals suffering from drug overdoses reduces activated charcoal adsorption capacity, it allows charcoal to be effective for a longer period [75].

Types of Adsorbed Drugs

A variety of drugs, phytotoxins, and dangerous compounds bind onto the activated charcoal surfaces, inhibiting their absorption through the gastrointestinal tract. In fact, it stops a potential enterohepatic and/or enteroenteric circulation as a secondary decontamination mechanism. The ability of activated charcoal to attach to the harmful chemical is determined by several parameters, including solubility, polarity, and ionization of the substance [76]. Non-dissociating compounds, such as mercuric chloride and iodine, are well adsorbed, although strongly dissociated salts, e.g. NaCl or KNO_3, are poorly adsorbed. Large, poorly water-soluble organic molecules, such as fatty acids, are more effectively adsorbed on activated charcoal than smaller molecules with polar substituent groups, as is the case of alcohols. Since pH may affect the process of ionization, salicylate is fully adsorbed at a low pH, when the drug is non-ionized, although the opposite is true in the case of basic compounds, such as aniline [77]. **Tables 2**, **3**, and **4** list compounds which, due to their physicochemical properties, undergo adsorption, are insufficiently adsorbed or are not adsorbed at all by activated carbon [56].

Activated Charcoal for Chronic Kidney Disease Patients

Patients with various stages of renal diseases are administered different forms of activated charcoal which is accompanied by reduced protein diets in order to control uremic symptoms. These occur when urea as well as other urine toxins bind to activated charcoal and are excreted with feces, forming a concentration gradient which allows the toxins to continue to diffuse [78]. Fur-

Table 2. Substances that are adsorbed on activated charcoal

Substance names
Amphetamines
ACE inhibitors
Antidepressants (except lithium)
Antiepileptics
Antihistamines
Aspirin salicylates
Atropine
Beta-blockers
Benzodiazepines (NB: somnolence)
Barbiturates
Quinine quinidine
Calcium-channel blockers
Dapsone
Chloroquine and primaquine
Digoxin
Digitoxin
Neuroleptics
Non-steroidal antirheumatics (NSAR)
Diuretics (especially furosemide torasemide)
Oral antidiabetics (especially glibenclamide glipizide)
Opiates dextromethorphan (NB: somnolence)
Paracetamol
Piroxicam
Tetracyclines
Theophylline

Table 3. Toxins that are adsorbed on activated charcoal

Toxin names
Amatoxin (death cap)
Aconitine (aconite)
Colchicine (autumn crocus)
Cucurbitacin (courgette, Cucurbitaceae)
Ergotamine, ergot alkaloids
Ibotenic acid, muscarine (fly agaric, panther cap)
Nicotine (tobacco)
Ricin (castor oil plant)
Strychnine (nux vomica)
Taxanes (yew)
Digitalis glycosides (foxglove)

Table 4. Substances that are insufficiently adsorbed on activated charcoal or not at all

Substance names
Alcohols (e.g., ethanol, methanol, and glycols [e.g., ethyleneglycol])
Inorganic salts (e.g., sodium chloride)
Metals and their inorganic compounds (e.g., lithium, iron, or other heavy metals [for instance lead or mercury])
Organic solvents (e.g., acetone, dimethylsulfoxide)
Acids and bases
Cyanides

thermore, other studies have shown that sorbents can help dialysis by removing waste products, such as urea, indoxyl sulfate, other urinary toxins, thereby improving the dialysis process [79].

Hypercholesterolemia Treatment

The studies demonstrated that activated charcoal is efficient in decreasing cholesterol levels. In one of the studies, seven patients with hypercholesterolemia were administered 8 g of activated charcoal three times daily for four weeks. Following this period, total cholesterol and Low-Density Lipoprotein-cholesterol levels in the blood were reduced by 25% and 41%, respectively [80]. In a three-week cross-over research, seven patients were administered charcoal at 4, 8, 16, or 32 grams per day, followed by bran. In a dose-dependent way, charcoal reduced the total and Low-Density Lipoprotein-cholesterol in the blood (maximum 29 and 41%, respectively) while increasing the High-Density Lipoprotein/Low-Density Lipoprotein cholesterol ratio (maximum 121%). Ten more patients with severe hypercholesterolemia were administered activated charcoal 16 g, cholestyramine 16 g, activated charcoal 8 g plus cholestyramine 8 g, or bran daily for three weeks in a random order. The Low-Density Lipoprotein-cholesterol total concentrations were reduced by charcoal (23% and 29%, respectively), cholestyramine (31% and 39%), and their combination (30% and 38%). Activated charcoal, cholestyramine, and their combi-

nation enhanced the High-Density Lipoprotein/ Low-Density Lipoprotein ratio from 0.13 to 0.23, 0.29, and 0.25, respectively [81].

Preventing Gas and Bloating

The evidence with regard to the effectiveness of activated charcoal in decreasing lower intestine gas and symptoms is conflicting. A double-blind clinical investigation was conducted which included two population study groups: in the United States (n = 30) and in India (n = 69), which were different in terms of eating habits as well as gut flora ecology. Breath hydrogen levels were tested using lactulose as the substrate to quantify the quantity of gas produced in the colon. In both groups, activated charcoal significantly decreased hydrogen levels in the breath and considerably reduced symptoms of bloating and stomach cramps resulting from gaseousness in both groups [82]. After consuming a gas-producing meal, the effectiveness of activated charcoal in treating intestinal gas was investigated. The number of flatus occurrences, as well as the levels of hydrogen in the breath, were also counted. Orally administered activated charcoal was found to be effective in preventing the substantial increase in the frequency of flatulence, and elevated breath hydrogen concentrations which occurred following gas-producing meals in these studies [83]. Moreover, the capacity of activated charcoal to suppress intestinal gas production was tested in vitro, as well as in vivo. Human fecal homogenates were involved in the in vitro investigations, which were incubated with or without extra carbohydrates. In all of the trials, the activated charcoal treated homogenate produced and consumed hydrogen, as well as carbon dioxide at similar rates as the untreated control group. Following a baked beans meal, a double-blind assessment of hydrogen and flatus excretion was used to investigate the effect of activated charcoal on gas generation in vivo. In patients who were administered 16 capsules of activated charcoal (4 g) vs. the placebo, no significant difference was observed in breath hydrogen content or in the occurrence of flatulence. In vitro and in vivo tests revealed that activated charcoal did not affect gas production [84]. Furthermore, the fecal release of intestinal gases was assessed prior to and following the charcoal treatment in five healthy individuals who voluntarily ingested 0.52 g of activated charcoal four times per day for the period of one week. Additional investigations were conducted in vitro aiming to compare the binding capability of charcoal to the sulfur gas generated by feces which would account for the in vivo results. According to the study results, activated charcoal ingestion did not cause a substantial reduction in the fecal discharge of any sulfur-containing gases, nor did it affect the total fecal gas release, or abdominal discomfort. In fact, as the in vitro investigations indicate, inability of the ingested activated charcoal to inhibit sulfur gas escape may stem from the saturation of binding sites of activated charcoal during passage through the intestine [85].

Reducing Body Odor

The potential of activated charcoal in wound treatment is related to its ability to adsorb gases. Activated charcoal is increasingly used to control smell, and it is particularly helpful in the treatment of fungating lesions [86]. The statistical study revealed that employing activated charcoal alone or in combination with soda-bicarbonate significantly reduced the odor. The study suggested using activated charcoal with or without soda bicarbonate as a cost-effective method to decrease the unpleasant odor accompanying severe skin loss [87]. Interestingly, activated charcoal was found to reduce the malodor of flatus in dogs by modifying the generation or availability of hydrogen sulfide in the large intestine, according to a study [88]. Leg ulcers are usually accompanied by odor, thus, reducing odor constitutes an adequate therapy. It was observed that activated charcoal dressing for malodor helped two individuals suffering from leg ulcers [89]. In addition, activated charcoal lowers healing time and eliminates bacterial barriers, according to an exploratory clinical trial aimed at determining the efficacy of an activated charcoal silver dressing in reducing the number of bacteria in chronic wounds with no clinical symptoms of local infection [90].

Treating Wounds

Zorflex is a brand-new antibacterial dressing composed entirely of activated charcoal fabric.

It draws bacteria to the surface and binds them there, thus allowing their removal when the dressings are changed. No known side effects have been demonstrated and, therefore, such dressings can be used for short, or extended periods of time. The activated charcoal cloth treatment was used to treat patients suffering from severe chronic venous leg ulcers, prone to recur in four case studies [91]. Clinical evidence suggests that employing activated charcoal impregnated with silver in the course of chronic wounds treatment, even at the debridement stage, could be beneficial. This process may be helpful in the removal of fluids and toxic substances which delay healing [92].

Use as Dentifrices

Toothpastes with activated charcoal are gaining popularity, despite the lack of evidence in terms of their safety in individuals with erosive tooth wear. Nevertheless, the use of such toothpastes did not put these persons at any greater risk [93]. Moreover, in a study regarding tooth decay, activated charcoal was found to be a deterrent to dental caries [94]. Activated charcoal toothpaste and powders are popular oral hygiene solutions for brushing teeth and removing extrinsic stains, and are thought to become more commonly used in various countries worldwide [95]. In fact, the charcoal-containing dentifrices were abrasive within permitted limits set by the ISO and did not adsorb fluoride [96]. However, another study indicated that the use of an activated charcoal-based product, described as a natural whitening agent, before brushing teeth with toothpaste is not only ineffective in terms of changing the color of the teeth, but may also result in enamel surface changes [97]. According to a recent review study, data to support the claim that charcoal and charcoal-based dentifrices are safe and effective are inadequate [98].

Conclusions

Adsorbents play a vital role in removing the effects of poisoning due to an overdose of drugs. One of such adsorbents, commonly used in the medical field, is activated charcoal. It has a substantial surface area, which renders it to be an effective adsorbent, it is also inexpensive and can be obtained from a variety of sources. Activated charcoal has a wide range of medical applications extending beyond the treatment of poisoning. It is used for hypercholesterolemia, in gas and bloating prevention, reducing body odor, treating wounds, as well as in dentifrices.

Acknowledgements

The authors would like to acknowledge the contribution of the University Of Anbar (www.uoanbar.edu.iq) via their prestigious academic staff in supporting this research with all required academic support.

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

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JMS Journal of Medical Science

Modern diagnostics in IgE-mediated cow's milk allergy

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DOI: https://doi.org/10.20883/medical.e690

Keywords: component-resolved diagnostics, cow's milk allergy, milk proteins

Published: 2022-06-30

How to Cite: Matysiak J. Modern diagnostics in IgE-mediated cow's milk allergy. Journal of Medical Science. 2022;91(2);e690. doi:10.20883/medical.e690

ABSTRACT

Cow's milk allergy (CMA) is the most common food allergy in infants and young children. Allergic reactions may vary from mild to severe, such as an anaphylactic shock. In the case of a suspected CMA diagnosis, skin prick tests (SPT), immunoassays of specific IgE (sIgE) in blood serum (in vitro tests) and oral food challenge (OFC) tests can be performed. SPT wheal diameter and the level of serum specific IgE to milk do not correlate with the severity of clinical symptoms, and the OFC procedure is frequently difficult or even impossible in practice. Therefore, component-resolved diagnostic (CRD) tests are a new diagnostic tool, which allows a better correlation of laboratory test results with the observed clinical symptoms as well as identification of the triggering allergens.

Introduction

Cow's milk allergy (CMA) is the most common food allergy in infants and young children [1]. According to various sources, the incidence reaches up to 7.5% [2–4]. In Poland, CMA affects between 0.5 to 4.8% of infants, although this food allergy occurs also in even 1% of adult patients [5]. Moreover, milk allergy is one of the major causes of anaphylaxis not only in the pediatric population, but also in adults [6]. It should be noted that epidemiological data regarding CMA prevalence highly depends on age, geographical region and the methodology of the diagnosis. The results of a pan-European Euro-Prevall birth cohort study constitute an example of these relations. The study has shown that diagnostics based on the gold standard – i.e. the food challenge, confirms the presence of CMA in less than 1% of children up to 2 years of age, and the occurrence of this allergy differs in European countries – the highest in the Netherlands and the United Kingdom (about 1%), and the lowest in Lithuania, Germany and Greece (less than about 0.3%) [7]. The symptoms of CMA may be associated with the skin, the gastrointestinal tract, the respiratory system, the cardiovascular system and the nervous system. Allergic reactions can vary from mild to severe, such as an anaphylactic shock [8].

Diagnostics tools in CMA

The first step in the allergy management is always a detailed interview and a clinical examination. In case of CMA suspicion, skin prick tests (SPT), immunoassays of specific IgE (sIgE) in

blood serum (in vitro tests) and oral food challenge (OFC) tests can be performed.

Skin prick tests (SPT) and sIgE determination in the blood comprise the first-line tests. Both methods demonstrate good sensitivity in the IgE-mediated CMA, but low specificity. Similarly to SPT, the determination of milk sIgE in the blood serum is associated with a high rate of false-positive results. Nevertheless, both of these methods constitute poor predictors of the severity of allergic reactions [9]. According to the data, the SPT wheal diameter and the level of serum specific IgE to milk do not correlate with the severity of clinical symptoms [2].

The key element in the diagnosis of food allergy is an attempt to eliminate a given food product, with a subsequent gradual introduction of it into the patient's diet. The diagnostic elimination diet should last for 2–6 weeks (usually 4–6), so that the patient does not present with any symptoms related to the food allergy [10]. After a few weeks, a gradual introduction of the food associated with the suspected allergy into the diet in increasing quantities occurs. This diagnostic procedure is commonly known as the oral food challenge test (OFC). OFC can be performed openly – the patient and the physician know what product is introduced into the diet, or as a blinded method. In the blinded challenges, either the patient (single-blind), or both the patient and the medical professionals (double blind) do not know whether the "real" test food, or the placebo is consumed. The double-blind, placebo-controlled food challenge (DBPCFC) is still a gold standard in food allergy diagnosis [11], although it is rarely performed in the clinical practice – usually only in research studies. OFC is very efficient in cases when the medical history and allergy tests results (skin and serological), are inconclusive. It verifies the actual allergic reactions to a given food allergen, and it is possible to differentiate between immediate and delayed reactions. Moreover, it is the only diagnostic method of non IgE-mediated allergy. Nevertheless, performing the OFC procedure in practice is frequently difficult due to the need to cooperate with the patient (or his parents) and the fact that it is time-consuming (an elimination diet must continue for several weeks before the food challenge). Additionally, in the case of severe allergy symptoms in the medical history, it must be performed at a hospital, which also limits the availability of this diagnostic method [12]. Moreover, severe anaphylaxis due to a suspected food allergen, is a significant contraindication to OFC, in view of the high risk of life-threatening reactions [10, 13].

Component-resolved diagnostics

Currently, the significance of the component-resolved diagnostics (CRD) in allergology is continuously increasing, particularly in terms of food allergy [14, 15]. SPT and the determination of sIgE in the blood serum allows the detection of the specific IgE against the whole extract, and in turn, each extract comprises a mixture of many allergens. However, the evaluation of patients' reactivity to the whole food extract is currently insufficient. Therefore, diagnosis based on allergenic components should be the basis for the diagnosis of IgE-mediated allergy [16, 17]. The allergen component is a protein constituting a fragment of the allergen extract with allergenic properties. The allergen component includes epitopes with either a linear, or conformational structure, and sIgE levels for individual allergenic molecules, or epitopes of allergens, can be detected by using CRD [18, 19].

CRD tests use a single, natural allergen isolated directly from the source, or an artificial recombinant allergen [20].

The European CRD molecular allergology user's guide indicates when to perform the component-based diagnostics [21]:

- inconsistency between the interview and the results of SPT and sIgE tests,
- inconclusive history, as well as clinical symptoms and tests results,
- allergy to one or more food allergens,
- coexistence of allergy to food and inhaled allergens,
- idiopathic anaphylaxis.

CRD technology improves the quality of life, since these methods allow for differentiating the cross-reactions from the real source sensitization and for the identification of the triggering allergens [22]. Additionally, it contributes to the optimization of the elimination diet and to enhanced identification of the patients requiring adrenaline [23].

CMA is one of the main food allergies where tests based on allergenic components should be performed in the clinical practice.

Cow's milk proteins

Milk is a mixture of many proteins. The latest data indicate that milk is a source of over 3100 different proteins [24]. The most important of which are the following allergens:

Casein (Bos d8) constitutes 80% of milk proteins, it consists of several fractions: α, β, γ and κ-casein (the most thermostable fraction). Sensitization to α fractions is the most frequent one, to κ fractions is the rarest. Casein is resistant to high temperatures and digestive enzymes, therefore, it is one of the most common triggering factors of anaphylaxis. Moreover, its resistance to high temperatures renders it a major cheese allergen. Beta-casein is present in A1 and A2 types. During A2 type digestion, peptide beta-casomorphin-7 occurs and is associated with gastrointestinal symptoms similar to lactose intolerance [25]. Casein is an important marker of persistent allergy to cow's milk proteins. Studies have demonstrated that basal levels of sIgE for both milk and casein may contribute to identifying the patients who may develop tolerance to milk [26].

Whey proteins – beta – lactoglobulin – **BLG** (Bos d 5), alpha- lactoalbumin – **ALA** (Bos d4), bovine serum albumin – **BSA** (Bos d 6), bovine immunoglobulins and bovine lactoferrin – constitute 20% of milk proteins and they are thermolabile at the temperature of 120°C (120°C for 20 minutes inactivates all whey proteins). Only bovine serum albumin is inactivated during cooking (already at 70–80°C). It should be emphasized that β-lactoglobulin sensitizes up to 80% of patients with whey proteins allergy, and it is not present in the human milk.

Casein, BLG and ALA comprise the major milk allergens and co-sensitization to these components is common [27]. Nevertheless, some patients are sensitized only to minor allergens – e.g. allergy to BSA is independent of sensitization to the other milk proteins. According to the recent studies, over 90% of children with CMA demonstrate sensitivity to caseins, between 35 and 61% to whey proteins [28].

Three CMA phenotypes can be distinguished, depending on the tolerance of baked and non-baked milk: reactive to baked milk, non-reactive to baked milk and non-reactive to non-baked milk [6, 29]. In fact, baked-milk intolerance phenotype is associated with casein allergy. The studies found that patients with this phenotype produce sIgE targeting against sequential milk proteins epitopes (mainly casein). This type of CMA is associated with severe clinical reactions to milk. In contrast, patients with detected sIgE against conformational epitopes showed tolerance to the extensively heated milk. Therefore, the inability to tolerate baked-milk products is a marker of the persistent CMA phenotype [28]. Patients who are non-reactive to non-baked milk are a phenotype with outgrew milk allergy.

Conclusion

Currently, it is possible to determine sIgE antibodies against casein, beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin and bovine lactoferrin, which allows for a better correlation of laboratory test results with the observed clinical symptoms, as compared to sIgE against the whole milk extract. In many cases it is possible to observe that, despite the negative sIgE result to the whole milk, increased levels of sIgE against milk components are found. Conversely, a negative result for the allergen components allows to exclude with a high probability an IgE-dependent allergy. However, many studies indicate that CRD tests are still not a perfect diagnostic tool in food allergy, and have not replaced OFC yet [17]. The data demonstrate that CRD have a high specificity but low sensitivity in the diagnosis of food allergy [14].

Concluding, CRD diagnostics provides the opportunity for a better diagnosis of patients with Ige-mediated CMA, although it does not replace other diagnostic methods, particularly OFC.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

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JMS Journal of Medical Science

A Thousand Words About Modern Medical Education: A Mini-Review Concerning the Theory of Education

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DOI: https://doi.org/10.20883/medical.e636

Keywords: Theory of Education, Bloom's taxonomy, FAIR principles, Gagné theory of learning, Harden's ten questions, Miller's pyramid, Outcome-based education, SMART method, SPICES model

Published: 2022-05-23

How to Cite: Al-Jarshawi MHA, Al-Imam A. A Thousand Words About Modern Medical Education: A Mini-Review Concerning the Theory of Education. Journal of Medical Science. 2022;91(2);e636. doi:10.20883/medical.e636

ABSTRACT

The present article represents a mini-review and a reflective essay concerning modern medical education methods, as well as ways to adapt them to medical education's local conditions (disciplines), including basic medical sciences. We introduced Gagné's theory of learning and other theories – Constructivist, Experiential, and Humanistic – followed by Dennik's "twelve tips" for effective learning and Harden's ten questions for curriculum development. Outcome-based education (OBE) was discussed and related to relevant concepts within Miller's pyramid and Bloom's taxonomy. Harden's SPICES model was emphasized concerning education strategies while discussing the assessment of learning (AoL), assessment as learning (AaL), and assessment for learning (AfL). Finally, the authors advise exploring the adaptation of modern education methods for a specific discipline of basic medical sciences – Human Anatomy – by incorporating the above-mentioned concepts and integrating different AfL and AaL assessment tools while conveying a graphical concept map for this scenario.

Introduction

Gagné (1965) pioneered the "conditions of learning" theory and recommended that each teacher's instruction should have nine ingredients: gain learners' attention, share objectives with learners, activate prior knowledge, present learning material, provide guidance, elicit practice, convey feedback, assess performance, and enhance retention and knowledge transfer [1, 2]. Dennik (2012) debated that the overarching worlds of views about how learning happens –

Constructivist/Experimental, Behaviorist, and Humanistic – possess common elements which could guide teaching-learning practices. Dennik's "twelve tips" require activating prior learning, consolidating existing knowledge, promoting social constructs, deploying active learning, reflecting on learning, providing relevant experience, and promoting self-assessment. Furthermore, it mandates to amass mental models and practical skills, engage in hypothesis-testing and action-planning, respect-accommodate learners' needs, and rapport with them [3]. In 1986, Harden developed a "ten-questions" approach for a curriculum development to identify learning needs, define outcomes, choose and organize educational contents, pick educational strategies, select teaching methods, design assessment, organize curriculum communication, exploit the environment, and manage the curriculum. Moreover, he also created a "design-down process" from exit outcomes to phase, course, and lesson outcomes [4].

Outcome-based education (OBE) relates to Miller's pyramid, Bloom's taxonomy, and the SMART method. In 1990, Miller proposed a hierarchical framework for assessing learners; knowledge is at the very bottom (leaner knows), then competence (knows how), performance (shows how), and action (does) [5]. Nevertheless, Witheridge (2019) argued that Miller's pyramid-related assessment tools, e.g., OSCE/OSPE, had limitations concerning assessing diagnostic reasoning; therefore, they proposed a revised pyramid which integrates cognitive skills, including diagnostic reasoning [6]. In 1956, Bloom developed three hierarchical models to classify learning objectives into three domains: cognitive, affective, and sensory [7]. Bloom's cognitive domain – which relates to Miller's pyramid – has a six-tier hierarchy of low-to-high order cognitive skills (knowledge, comprehension, application, analysis, synthesis, and evaluation) [8]. On the other hand, the SMART method aims at well-defined learning outcomes (specific, measurable, attainable, relevant, and time-bound) [8, 9].

In terms of the curriculum contents, it mandates three phases: before (prerequisites), during, and after (future learning), whereas teaching methods should correspond to high-retention rates within the learning pyramid, via which it is possible to progress from passive to active learning and from visual-auditory to kinesthetic learning experience (**Figure 1**). [4, 10]. In 1984, Harden pioneered the SPICES model for educational strategy, which refers to six elements, and each has a continuum, including student/teacher-centered, problem/information-based, integrated/discipline-based, community/hospital-based, electives/standard program, and systematic/apprenticeship-based [11].

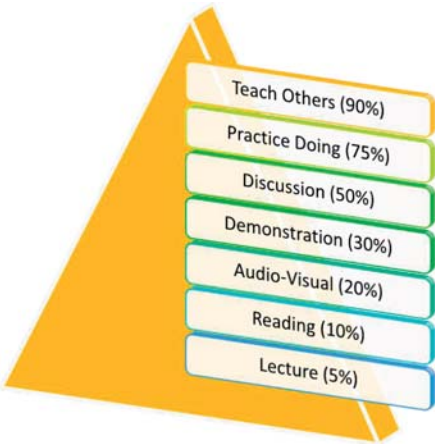

Figure 1. The learning pyramid: Teaching methods and retention rates

Learning assessment can be challenging, and teachers visualize it from learning outcomes to teaching activities to assessment via constructive alignment; in contrast, students' perspective is reversed [4, 5]. Instructive alignment relates to the "golden triangle" – objectives, assessments, and instructional methods promote educational outcomes [8]. Archetypally, assessment was an evaluation "of" learning (AoL); however, a programmatic assessment should be "for"/"as" learning (AfL and AaL) to steer learners towards maximum potential by evaluating learning dimensions to optimize learning and educational decision-making [4, 5, 12]. Schuwirth (2011) demanded novel psychometric models and human judgment for robust assessment; he also emphasized an "N:N relationship" instead of a "1:1 relationship" with regard to assessment instruments, i.e., an assessment tool should map, or inform on, more than one competency domain [13]. AfL evaluates competencies and identifies gaps and confusions; it can be diagnostic (map prerequisites), formative (guide-maintain regular learning), and summative, while AaL requires meta-

cognition with learners critically analyzing new information, relating it to their prior knowledge and experience, and investing it for future learning and practical application [14].

Harden and Laidlow (2012) emphasized assessment "as" learning by introducing the FAIR principles (**Figure 2**), in which a teacher should incorporate feedback to learners, conduct active learning, individualize the learning process according to learner's needs and abilities, and ensure relevant learning; these principles evaluate learners' competencies and deficiencies, identify best learners, motivate others, and promote teacher's competence [12]. In fact, FAIR's most challenging element is the individualization principle which relates to Guttman's scale according to which students vary in terms of their abilities to learn or to solve simple to complex problems[15]. AfL and AaL should accompany each tier of Miller's pyramid; educators must use factual tests (knowledge), clinical-based tests (competence), controlled environment tests (performance), and real-life tests or scenarios (action) [5, 6]. Practical assessments include OSCE/OSPE, MINI-CEX, 360-degrees assessment, and Portfolios, while written forms, addressing lower tiers of Miller's pyramid, include MCQs, true/false questions, matching/extended-matching, fill in the blanks, short-answer, and essay questions; nonetheless, each has advantages and disadvantages [14]. In 2010, Cook conveyed "twelve tips" for the holistic evaluation of educational programs – his third tip stresses the difference between assessment and educational program evaluation [16].

In 2003, Hutchinson debated that good educators can optimally maneuver their educational environment, while the environment and learners' interaction determine learners' motivations, tasks perception, and relevance. Thus, educators should maximize the physical environment and ensure adequate spacing-seating, comfortable climate, minimizing distractions/noise, and utilize audiovisual equipment [17, 18]. Learners' motivation is pivotal, and it relates to the educational environment and Maslow's hierarchy of needs – physiological needs (base), safety, belonging, self-esteem, and self-actualization (top) (**Figure 3**) [19].

As far as adopting modern medical education methods and techniques to a specific disci-

Figure 2. FAIR Principles for assessment as learning (AaL)

Figure 3. Maslow's hierarchy of needs

pline is concerned – human anatomy in the case of the present paper – the authors recommend implementing Harden's "ten questions", Gagné's recommendations on teachers' instructions, and OBE in congruence with Miller's pyramid and Bloom's taxonomy while utilizing the SMART and SPICES models customized for each institution's requisites (**Figure 4**). Furthermore, medical educators can aim for programmatic AfL and AaL by integrating FAIR principles and optimum maneuvering of the physical elements of the educational environment in accordance with Maslow's hierarchy of needs. Nevertheless, anatomy teachers may still experience difficulties within the holistic education process, due to the fact thatanatomy is an intricate subject which mandates phasic assessment (diagnostic, formative, and summative) and multi-mapping (N:N) of the competency domains, including theoretical and clinically-oriented knowledge, three-dimensional anatomical appreciation, cadaveric dissection or its virtual simulation, as well as cognitive-diagnostic reasoning in the clinical context. For instance, combing demonstrator-assisted cadaveric dissection with virtual simulations of anatomical models in our instructional design of teaching applied anatomy could draw on both constructivist views regarding learning, particularly Vygotsky's theory in working with learners at their zone of proximal development [20], and the behaviorist doctrines according to which learning happens only in the course of in situ observation, i.e., Bandura's cognitive theory of social learning [21].

Acknowledgements

Ahmed Al-Imam is a participant in the STER Internationalisation of Doctoral Schools Programme from NAWA Polish National Agency for Academic Exchange No. PPI/STE/2020/1/00014/DEC/02.

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
The authors self-funded the study. Ahmed Al-Imam received funding – a research grant – from statutory funding for young scientists in 2021–2022. However, he did not use the grant's money to fund or pay for its publication charges.

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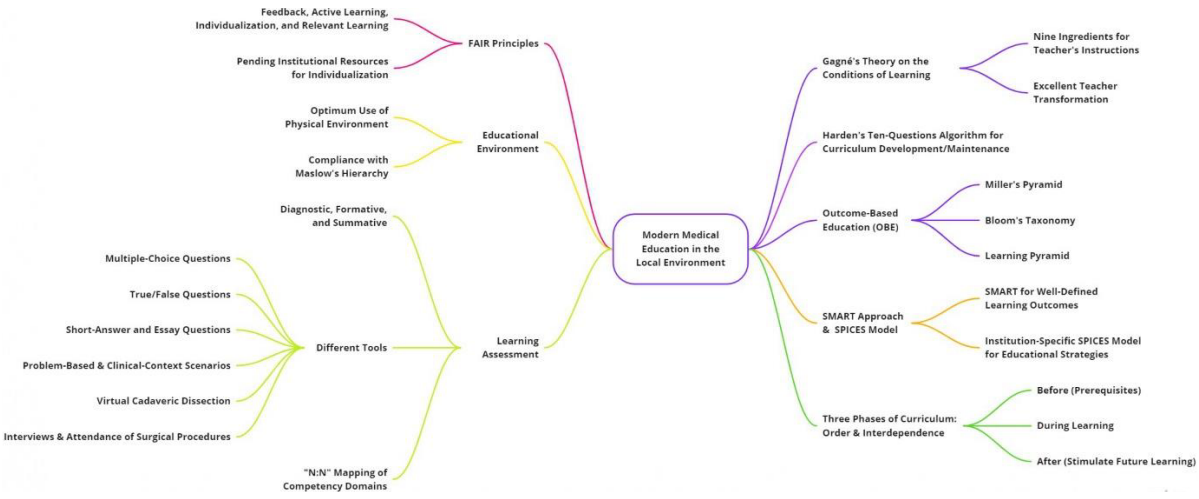

Figure 4. Concept map of modern education in local conditions

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E-scooters and the City – Head to Toe Injuries

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Keywords: electric scooters, accidents, head injury, TBI, road safety

Published: 2022-06-30

How to Cite: Kowalczewska J, Rzepczyk S, Żaba C. E-scooters and the City – Head to Toe Injuries. Journal of Medical Science. 2022;91(2);e672. doi:10.20883/medical.e672

DOI: https://doi.org/10.20883/medical.e672

ABSTRACT

In the recent years, electric scooters have become much more common. With an increasing number of drivers, the number of accidents and injuries involving e-scooters also was on the rise. The most common are head and neck injuries, including open wounds of soft tissues, traumatic brain injuries (TBIs), cranial bones fractures, and intracerebral hemorrhages (ICH). The second most frequent injuries involve both upper and lower extremities. Fractures of the spine and injuries to the thorax and the abdominal cavity are less common. Many injuries could be avoided by using helmets, not driving e-scooters under the influence of intoxicating substances, and systematizing the rules of e- scooters use among other road traffic users.

Introduction

The market has been overrun by companies offering rental of electric scooters, which caused a change in the urban micromobility. Accessibility, simplicity, affordable price and environmental friendliness have significantly contributed to the popularization of this mode of transport [1]. In Poland, the first e-scooter rental system via a smartphone application was introduced in Warsaw in autumn 2018, and subsequently also in other cities [1]. Nevertheless, as the number of users increases, so does the number of accidents and injuries involving these vehicles [1–3]. Research shows that since the introduction of e-scooter rental systems, the incidence of injuries resulting from accidents involving them has more than doubled; furthermore, the frequency of hospitalization in such cases has increased over threefold [4].

Risk factors

There is a number factors affecting the likelihood of an accident and injury related to the use of an e-scooter. The type of accident plays an important role, and the most dangerous are the least frequent ones, that is collisions with another vehicle, followed by hitting objects and falls, which are most common [5]. Usually, young men are the casualties of such accidents [6, 7]. The

most prevalent time for e-scooter related injuries is at weekends in the evening during the summer months [7, 8]. Another significant factors are risky activities, such as using the phone while driving as well as using the vehicle under the influence of alcohol, or intoxicants, such as cannabinoids [2, 9]. No requirement to wear protective helmets presents another vital aspect regarding the head injury risk. The percentage of e-scooter drivers using helmets rarely exceeds 5% [5]. Additional risk factors include high speed (the maximum permitted speed of e-scooters in Poland is 20 kilometers per hour; however, the engines used in these vehicles allows them to reach twice the maximum speed), tandem driving and driving on the sidewalk [1, 10].

Injury pattern

Head and neck injuries constitute the most common e-scooter related trauma [11, 12]. They occur in approximately 30–50% of all accidents involving e-scooters [11, 13, 14], which may be the result of the low percentage (about 4%) of users wearing protective helmets [14]. Many craniofacial traumas stem from inertly hitting the ground as a result of an unsuccessful attempt to prevent the fall. Moreover, high center of gravity of the scooter rider predisposes them to sustain head injuries [2]. In most cases, head injuries are minor, however, in approximately 25–30% of patients, open wounds of soft tissues require surgical intervention, around 15% develop TBI (Traumatic Brain Injury), about 10% have tooth fractures, and approximately 6% develop symptoms of concussion. About 2% of patients also sustain ICH (Intracerebral Hemorrhage) [2, 11, 12, 15]. The most frequently damaged areas of the soft tissues of the head include the forehead, scalp and chin. Soft tissue injuries, most often manifested in the form of lacerations and abrasions, can leave permanent esthetic defects on the skin of the face, despite being treated quickly and accurately. The midface (nasal bone, maxillary sinus, lateral wall of the orbit, zygomatic bone) is the most commonly damaged area in terms of the cranial bone fractures. Additionally, patients with fractures of the skull base might sustain cerebrospinal fluid leakage [2]. Patients, who have had a scooter accident often undergo imaging examinations of the head – about 20% of them show intracranial lesions, such as brain contusion, traumatic subdural hemorrhage, traumatic subarachnoid hemorrhage, diffuse TBI, epidural hemorrhage [2, 12, 15, 16]. Furthermore, a small percentage of patients require neurosurgical intervention, but many of them require outpatient observation. The individuals may need hematoma evacuation or intracranial pressure monitoring. The vast majority of the patients with head trauma were under the influence of alcohol during the accident, indicating alcohol consumption as a particular risk factor for head injury while riding an e-scooter [16]. There is a five-fold higher risk of TBI in patients who were under the influence of intoxicants during an e-scooter accident, and the risk of their hospitalization increased two-fold [17]. Most head traumas do not require extensive medical intervention, however, the long-term effects of the injuries cannot be assessed directly following the accident, in the emergency room. As a result of a short-term consciousness impairment, 40% of the patients will experience chronic headaches and fatigue [11, 14, 18]. TBI and ICH may also be associated with the occurrence of post-traumatic epileptic seizures of various nature which may take place at different times after the accident [19]. It is also vital to bear in mind the rarer cases of compression fractures of the spine and the occurrence of central cord syndrome [15, 16]. Nevertheless, head injuries, half of which are TBIs, are the most common cause of hospital admissions resulting from e-scooter accidents [20]. TBIs are also the leading cause of death among the young [21].

Except for the head injuries, limb injuries are the most common e-scooter related trauma [22, 23]. The very method of driving the vehicle contributes to this fact. The upright position and the higher center of gravity during sudden braking causes the user to be thrown over the handle bar, which results in limb injuries due to their protective positioning [2]. In the area of the upper extremities, injuries occur in 47–51% of cases, and the most common include lacerations, bruises and fractures in the distal parts of the wrist and the forearm [3, 5, 7, 23]. Injuries to the shoulder girdle e.g. ruptures of the ligaments and muscle attachments along with fractures of the scapula and clavicle, are also typical [24, 25]. In the area of the lower extremities, injuries occur in 36–48%

of cases, usually in the distal parts [3]. Fracture to the metatarsal bones and ankle sprains are frequent as a result of clumsy pushing, or sudden leg dropping from the vehicle [5, 7]. In the area of the lower extremities, fractures are most commonly found in the lower leg [23]. Additionally, fractures of the femoral neck, as a result of a fall, have also been reported [24]. Significant therapeutic problems, frequently requiring surgery, are injuries of the knee joint, damage to the bones forming the joint and the ligamentous apparatus [24]. Overall fractures occur in 18–31% of cases, and lower limb fractures more often require surgical treatment [5, 26]. Chest and abdominal injuries are relatively rare, however, there have been reports of rib fractures, pneumothorax, and damage to the structure of the lungs or liver [5, 7, 25]. Approximately 5% of patients require hospitalization, and 1% require intensive medical care [5]. Fatal accidents are rarely reported, although some studies indicate a significant underestimation in this regard [5, 6].

Conclusions

It is crucial that physicians working in trauma centers are educated regarding the typical injuries that e-scooters users may report. Furthermore, it is vital to develop detailed diagnostic protocols following the abovementioned accidents. It is essential to record events involving e-scooters and thoroughly research the frequency and characteristics of e-scooter related trauma in individual regions. The collection of this data will allow for a better understanding of injuries typical for users of such vehicles, which will result in the development of a diagnostic protocol after such accidents, as well as methods protecting against the consequences of road incidents for users of e-scooters. For the sake of safety, it is necessary to adapt the legal regulations related to the use of e-scooters and to create a road-friendly infrastructure to accommodate them. In addition, it is essential to raise awareness regarding the potential benefits of using helmets, with the emphasis on the danger of not using them, which could prevent numerous future injuries. It is also advisable to introduce appropriate rules for the use of scooters on pavements, in order to prevent potential accidents involving pedestrians.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

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