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RESEARCH ARTICLE

Going the Full Circle: Upgrading the Patient Field Chart and Tag for Electronic Mass Casualty Incidents Solutions in Romania

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Objective: Mass casualty incidents and disasters require functional and efficient patient data management systems, as well as smart interconnections with patient tracking applications. Various initiatives developed and tested patient field charts for large-scale events but there is no one definite general format accepted. The current research proposes an upgraded model of the official patient field chart issued by the Romanian Department for Emergency Situations in 2015 to be used for large-scale events. **Measures**: An upgraded model is created after a thorough content analysis, physical analysis, design upgrade and optimization process. Differences between the official and the upgraded model are measured and compared, and statistical computations are carried out. **Results**: The main distinctive features of the patient field chart are dynamic triage, unique code identification, QR visual codes, wireless tags and irreversible clear contamination status highlighting. The upgrade process results in almost doubling the available active area without the need to change the document size format of the product. Visual elements and features are included to optimize operation workflow. **Conclusions**: The upgraded model offers a variety of improvements for both the overall rescue effort as well as the end user of the product. It allows for previously unavailable features like unlimited dynamic triage and enables the use of electronic management solutions.

Keywords: patient field chart, triage tag, dynamic triage, mass casualty incidents, electronic management

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Introduction

Mass casualty incidents (MCIs) and disasters require functional and efficient patient data management systems, as well as smart interconnections with patient tracking applications [1, 2]. While tracking features are mainly provided on a daily basis by medical transport systems in place, prehospital patient data management during large-scale events may be troublesome given the great amount of data to be input in a very compressed timeframe [3, 4].

The particularity of this type of events consists of the lack of usefulness of certain elements contained in the usual prehospital data charts and the critical necessity of other missing specific elements [5]. To this end, MCIs patient field charts (PFCs) need to have a dedicated identification code, a coding system for the severity of injuries at the time of triage or later on as the patient moves along the rescue chain, and contamination status indicators.

While regular patient data charts allow for more elaborate enunciations in terms of patient history, physical examination and diagnosis, it is essential for the information to be essentialized during MCIs data documentation. One solution to maximizing efficiency and preserving detail is to take advantage of visual elements instead of plain text and optimize the chart content so that the workflow is fluent and intuitive [6, 7].

The development process of a dedicated PFC to be used during large-scale events adheres to general guidelines for this purpose. The requirements are largely accommodating and do not affect the legal quality of the document, but ensure an optimal integration of such forms into the healthcare process and set up a professional standard [8, 9]. Various initiatives developed and tested PFCs for MCIs but there is no one definite general format accepted [10].

The current research proposes an upgraded model of the PFC dedicated for large-scale events to be used by the Romanian authorities starting from a pre-established official model issued by the Romanian Department for Emergency Situations in 2015. The aim is to optimize the existing format and provide modern capabilities in terms of large-scale events management.

Materials and methods

Running a new optimized design for a PFC requires a methodic approach starting with a careful evaluation of the existing model. This mainly requires four large steps: a content analysis, a physical analysis, upgrading the design and optimization of the end product.

The Process

The first step – the content analysis – is necessary in order to identify the overall structure of the document and the elements of each section. Later on, each element is analyzed for the necessity of its presence and any required modifi-

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cations in terms of expanding or collapsing. This may in turn give rise to supplemental elements or exclude some of them. At this point, any additional elements are taken into consideration in order to obtain a complete desired content. This may generate the need for new sections.

The second step – the physical analysis – requires listing the physical elements of the product. This depends on the necessary and desired properties and its functionality.

The third step – upgrading the design – is the main step of the process. It unifies the content and physical elements so that the product expresses the strategy of its use while accounting for an improved ease of use in terms of straightforwardness and fluency.

The last step – optimization of the product – is an absolute must in order to ensure the uniqueness of each section by its graphical format, a proper choice of colors and elements sizes with respect to their importance and fill-in sequence, a unitary approach of graphical properties and imagery, and maximum useful coverage with minimum dead space.

Content Analysis

The existing model is a full Letter (US) format – 8.5 x 11 inches, that is 215.9 x 279.4 mm, a canvas size with an aspect ratio and absolute values very similar to the European A4 sheet (210 x 297 mm). The design does not feature any printer margins and is a two-sided document. Since the document in use is printed on a standard A4 sheet, we are going to consider a largely accommodating printing margin of 5 mm on each side for all future documents, which makes up a surface of 574 cm² (200 x 287 mm) available for full printing on each side.

When scaling to this format, the existing model has a printed surface of 377.3 cm² (200 x 188.65 mm) on the front page and 380.24 cm² (200 x 190.12 mm) on the back page, the remainder being blank (Fig. 1)

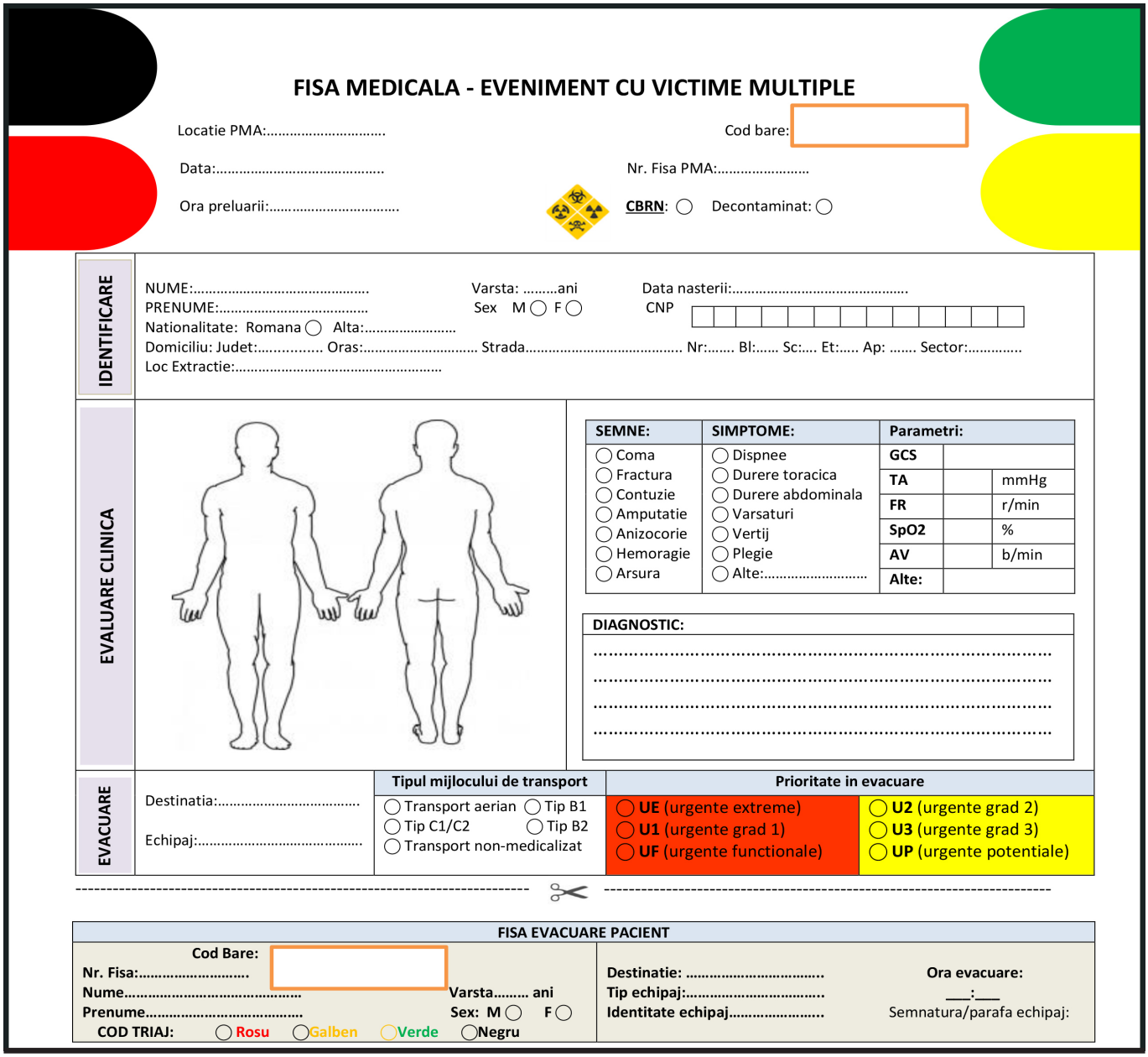
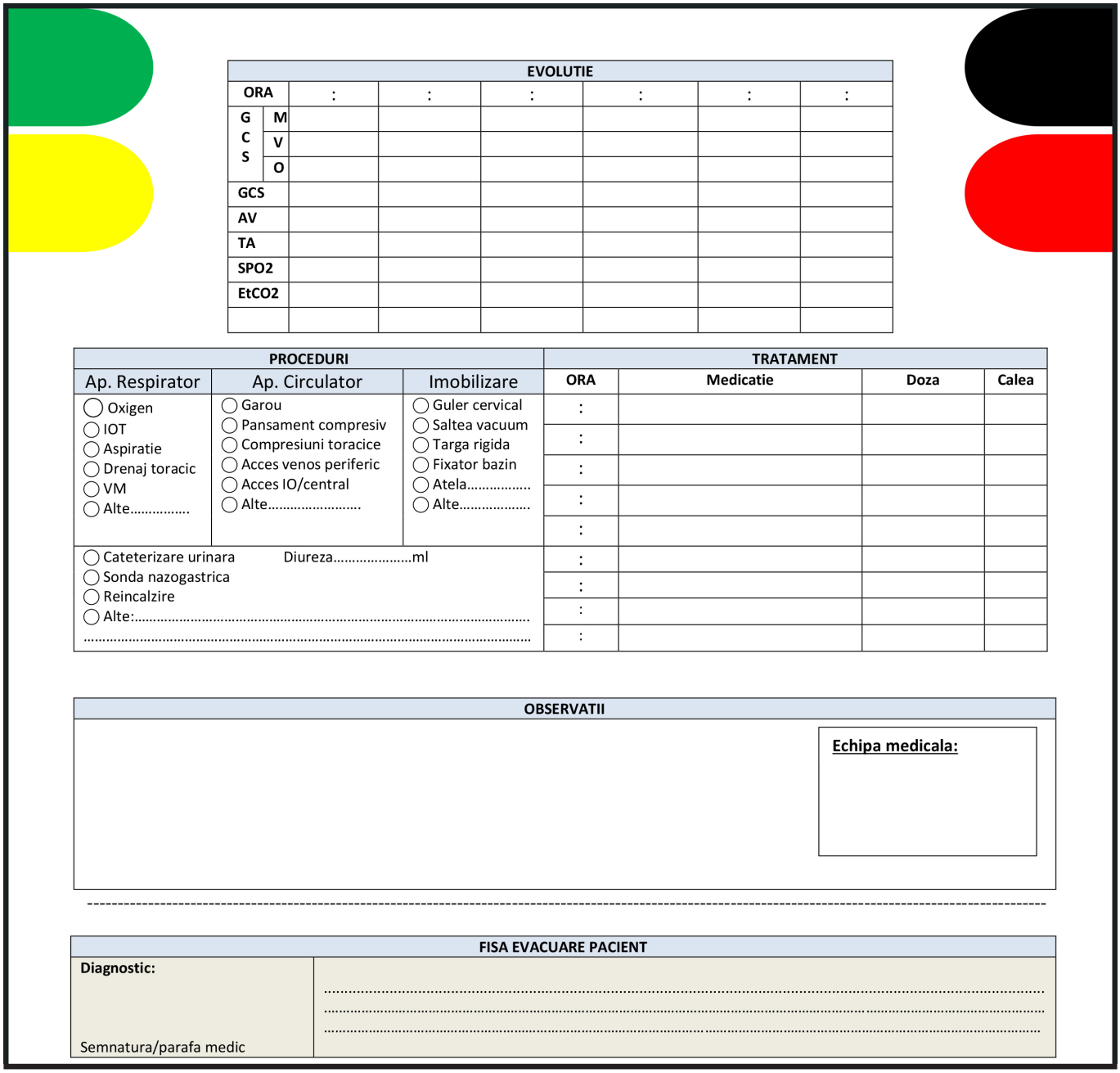


Fig. 1. The front side of the official format issued by the Romanian Department for Emergency Situations in 2015



EVOLUTIE

ORA		:	:	:	:	:	:
GCS	M						
	V						
	O						
GCS							
AV							
TA							
SPO2							
EtCO2							

PROCEDURI

Ap. Respirator	Ap. Circulator	Imobilizare
◯ Oxigen ◯ IOT ◯ Aspiratie ◯ Drenaj toracic ◯ VM ◯ Alte.....	◯ Garou ◯ Pansament compresiv ◯ Compresiuni toracice ◯ Acces venos periferic ◯ Acces IO/central ◯ Alte.....	◯ Guler cervical ◯ Saltea vacuum ◯ Targa rigida ◯ Fixator bazin ◯ Atela..... ◯ Alte.....

◯ Cateterizare urinara Diureza.....ml
◯ Sonda nazogastrica
◯ Reincalzire
◯ Alte:.....
.....

TRATAMENT

ORA	Medicatie	Doza	Calea
:			
:			
:			
:			
:			
:			
:			
:			
:			

OBSERVATII

Echipa medicala:

FISA EVACUARE PACIENT

Diagnostic:

.....
.....
.....

Semnatura/parafa medic

Fig. 2. The back side of the official format issued by the Romanian Department for Emergency Situations in 2015

The lower edge on each side of the document is fitted with a dotted line in order to separate the main part of the PFC from the evacuation note that is left on site at the Advanced Medical Post (AMP). Thus, the main part, that stays at all times with the patient, is printed on the front page on the upper 160.71 mm side while on the back page it is printed on the upper 162.12 mm side.

The existing official concept features a title a brief landmark area for date, time, location, contamination status and PFC ID, an area for the severity code elements (green, yellow, red and black) divided in two with a half on each upper corner, a patient identification section and 6 medical sections. The bottom part is occupied on each page by the removable evacuation note. The main part of the front page includes the title, the landmark area, the PFC ID, the severity code elements, the patient identification section and the first two medical sections i.e. Clinical Evaluation and Evacuation. The main part of the back page includes the mirrored severity code elements and the remaining 4 medical sections: Evolution, Procedures, Treatment and Observations. This last one contains an area dedicated to listing the medical team responsible for the patient.

Physical Analysis

Although the concept initially required weatherproof materials to be used in the making of PFCs, in practice it is printed on regular 80 grams plain paper. In addition, there are no self-adhesive materials used for applying a barcode label or any other sort of inclusions. Basically, the concept only requires a regular writing tool as any other medical document that can be found in a hospital setting. There

is no dedicated area for an attachment mechanism of the PFC to the patient.

Design Analysis

In terms of functionality, the design lacks dynamic triage and does not provide a patient bracelet for tracking purposes. A severity code is assigned only once – at the beginning of the rescue chain on site – by removing the finger-shaped side elements that do not correspond to the correct severity code, leaving only the proper one in place. Two barcode labels are necessary – one at the top of the PFC and the other on the evacuation note – acting as a pair, however it is not clear where do these labels come from or how and when are they generated. Removing the evacuation note is simply done by folding it and ripping it off. The PFC is designed to accompany the patient at all times, whereas the evacuation note is left at the evacuation point of the Advanced Medical Post.

Concept Upgrade

Upgrading the current concept revolved around three targets: enabling dynamic triage, increasing and improving visibility of all areas and the overall usage of the PFC, and bringing the concept up to speed with current and future electronic management solutions for mass casualty incidents.

Enabling dynamic triage involves the use of mobile elements. Each mobile element must possess its own severity code that can be highlighted at any given time depending of the severity status of the victim, and most importantly – it must be visible from a distance. In addition, changing the severity code has to be allowed any number of times and in any required sequence, including reverting to a previous code. The mechanism must allow a rapid change in severity status while minimizing the risk of accidental changes or element detachment.

Increasing and improving visibility of all areas impacts on the product form factor and modifying the general structure so that any available previously unused space is reconfigured so that the active surface covers as much of the printed space as possible while also increasing the visibility of text and figures and maximizing fill-in areas. Any free space that cannot be used as an active surface may be used for brief and clear instructions for the personnel using the PFC. Applying a proper color strategy by separating adjacent sections using different colors and assigning adequate color intensities to the different regions of the PFC in respect to their importance and sequence of use is part of the visual optimization process. Dedicated areas for contamination status and visual and electronic coding are assigned to the identification section.

Integrating the upgraded concept within electronic management solutions implies embedding of wireless (NFC/RFID) tags as well as visual codes that may be typed or scanned by cameras or industry scanners. Simplifying the unique identification of each PFC requires redundant strategies while ensuring there are no multiple identifiers for a given PFC.

Using a patient bracelet for ensuring patient identification and tracking at all times requires a largely simplified structure with written identification elements and any other essential information, as well as visual codes with or without wireless tags. The functionality of the bracelet is in complementary use with the PFC.

Upgrading the material list is essential since the selection must account for mobile elements, detachable labels and embedded tags as well as the use of the product under varying environment conditions.

The upgrading process starts with establishing the new design and its functionalities, continues with redefining the overall structure and the included content and ends with a careful and exhaustive optimization process.

Evaluation

The component sections of both models are precisely measured using professional software – CorelDraw X8 (version 18.1.0.661, Corel Corporation, 2016). Measurements are reported as square centimeters and values are rounded to two decimals. Differences are noted and the content is analyzed. Statistical analysis is carried out by applying chi-square tests using STATA/MP 14.1 (StataCorp LLC). Statistical significance threshold was set to a p-value of 0.05 for a confidence interval of 95%.

Results

Concept Upgrade

The upgraded concept contains the PFC and the patient bracelet as separate but complementary items. Each pair is linked by unique alphabetical codes, visual QR codes and wireless NFC/RFID tags, all with a unified correspondence.

The main distinctive features of the PFC are dynamic triage, unique code identification, QR visual codes, wireless tags, irreversible clear contamination status highlighting and the AMP entry point note (Fig. 3, 4).

Design Analysis

Dynamic triage works by flipping color-coded magnet fastening evacuation notes from the front side to the back side of the tag and vice versa and (un)covering the corresponding triage color on both sides. There are four AMP evacuation notes available – one for each severity code, and a similar sized AMP entry point note – all located on the front left hand side of the PFC. All five notes are detachable and are used in order to keep patient records at the entry and exit points of the AMP. The top note is removed at the triage (entry) point and only one of the four evacuation notes – the one with the current severity code – is removed at the exit point.

Each PFC and patient bracelet pair (Fig. 5) is assigned a unique 4-character ID code. This makes up a total of 439400 unique IDs. There is a total of 9 copies of the

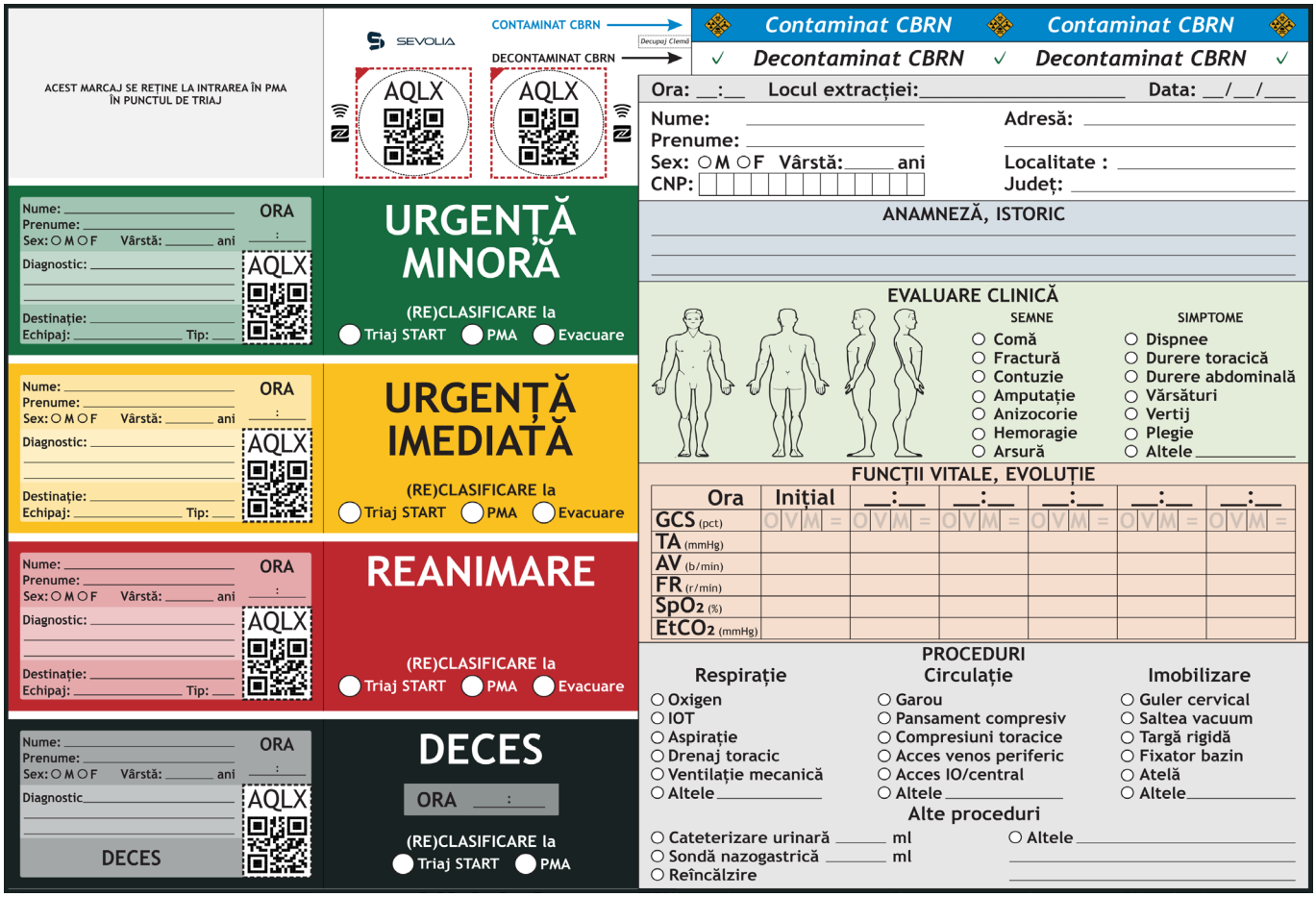


Fig. 3. The front side of the upgraded model (design)

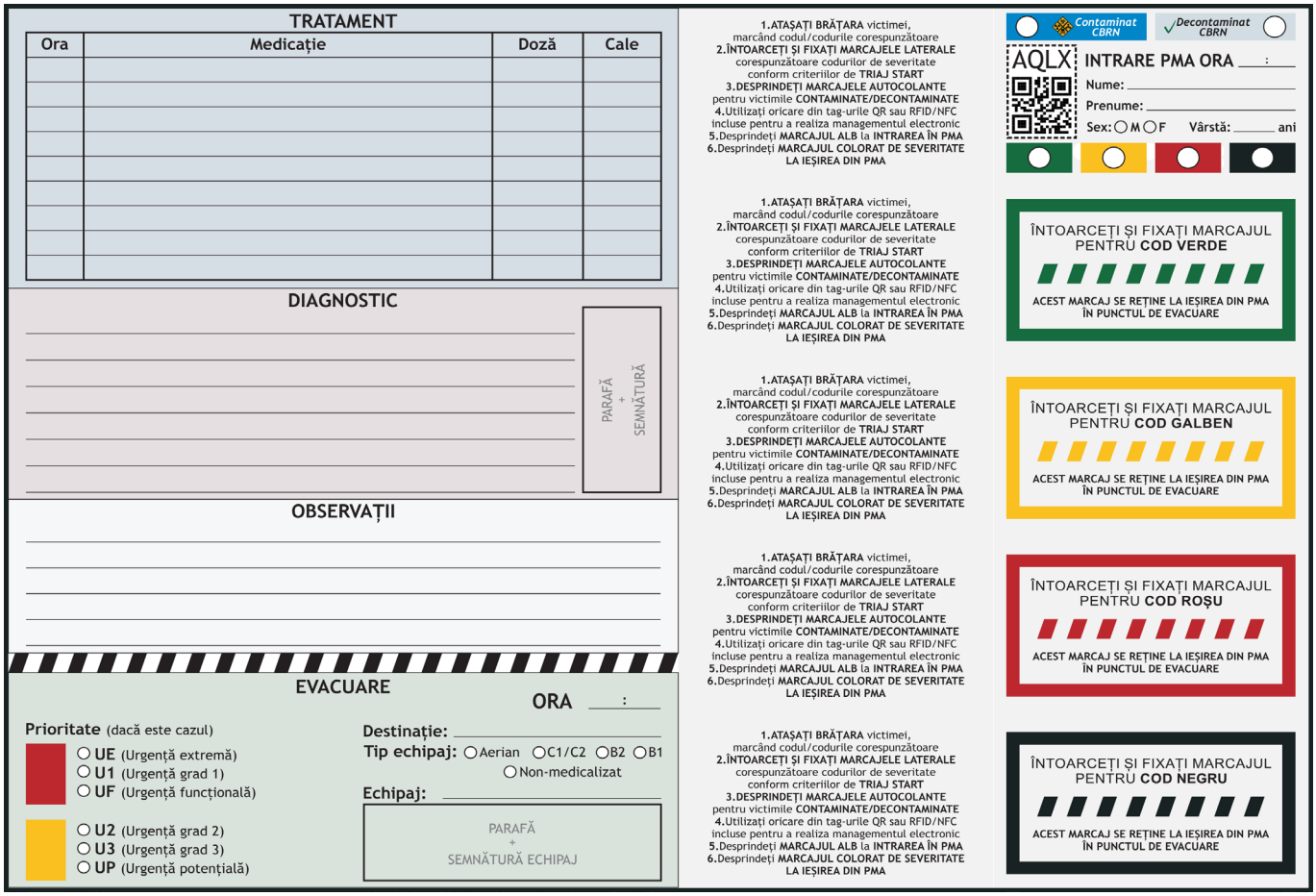


Fig. 4. The back side of the upgraded model (design)

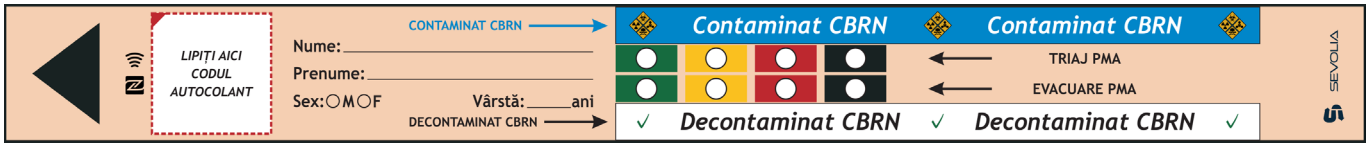


Fig. 5. The patient bracelet paired with the upgraded model (design)

assigned code and all of them are initially located on the PFC. Two of them can be found on larger self-adhesive labels at the upper left corner on the front side of the PFC. This allows for any bracelet to be linked to any new PFC. One of these two can be removed and then stuck on the corresponding space at the left edge of the patient bracelet. The remaining one is for applying the label onto hospital documents. The area remaining beneath the self-adhesive labels is printed as well containing the same copies.

There are two markings for the contamination status, "CBRN contaminated" and "CBRN decontaminated", which may be rendered visible by peeling off the corresponding coverings at the front top edge of the PFC. The same functionality is applied to the corresponding patient bracelet.

When in use, the front and back sides of the PFC are a mixing of the front and back sides of the designed model, lacking removed elements at different times of the rescue chain. The patient bracelet will also have its ID label applied. A proper example of the PFC and patient bracelet for an uncontaminated patient at the time of secondary triage is depicted below (Fig. 6, 7, 8).

The PFC may be tied to the patient using a cord or any other binding mechanism passed through the clamp clipping at the center top.

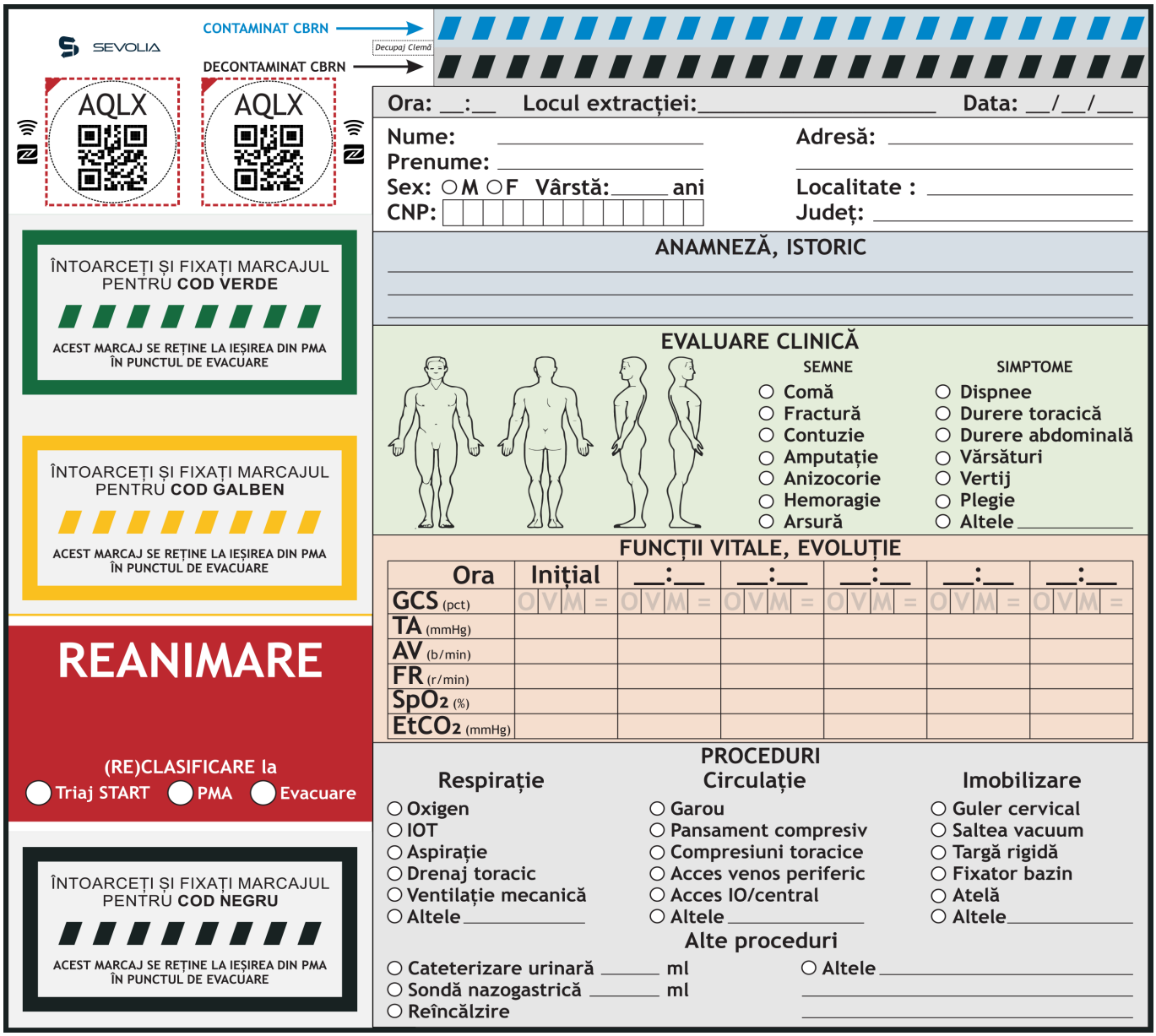


Fig. 6. The front side of the upgraded model (in use)

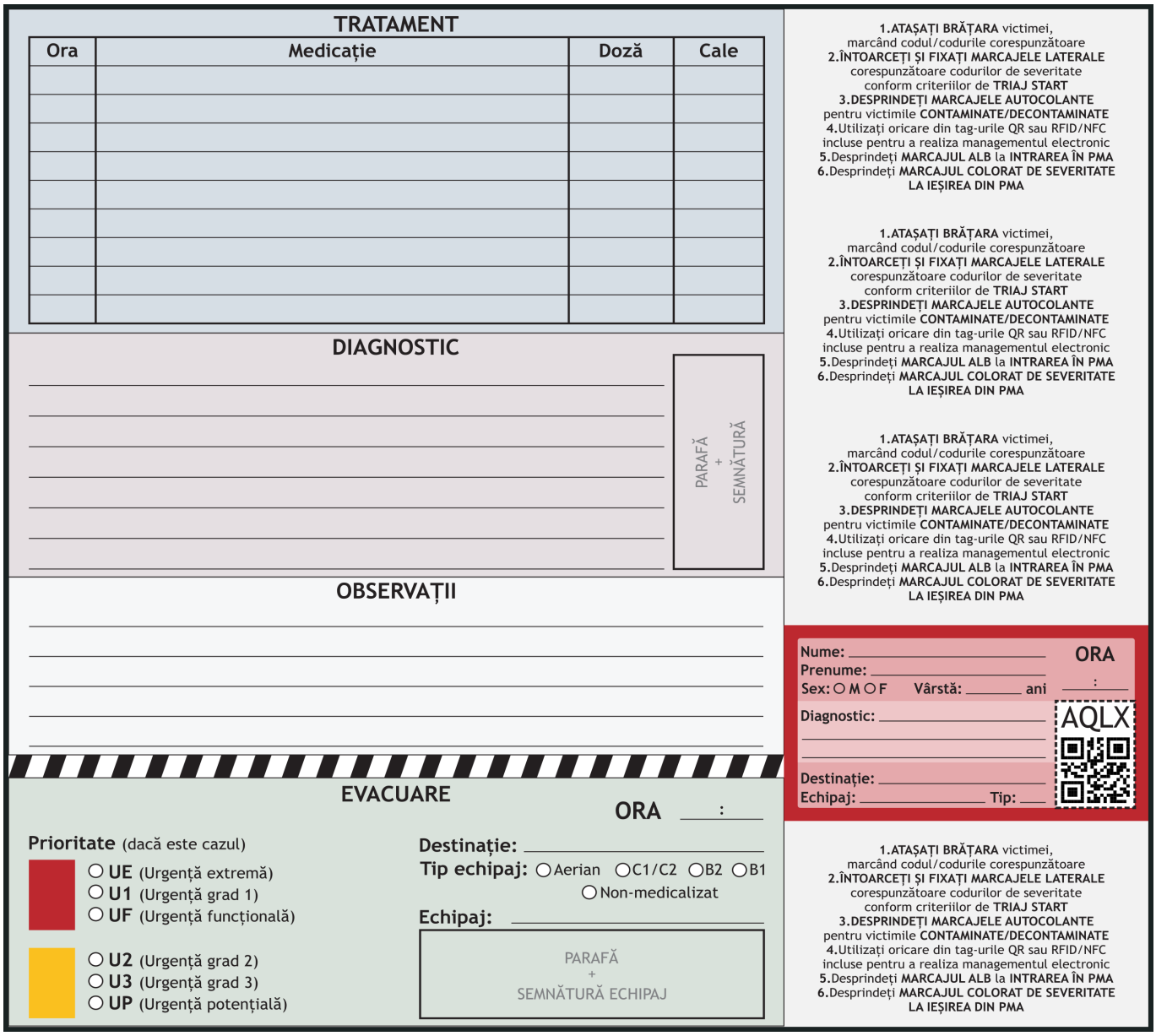


Fig. 7. The back side of the upgraded model (in use)

Removing the proper labels at the entry and exit point of the AMP respectively allows for independent patient inventories at these locations. The entry label contains the patient's name, sex, age, initial severity classification and contamination status as well as the 4-character ID and its corresponding QR code. The exit label has a colored background according to the severity code at the moment of the evacuation from the AMP, containing the patient's name, sex, age, destination, pickup crew identification and type, diagnosis and the 4-character ID and its corresponding QR code. Sample entry (Fig. 9) and exit labels (Fig. 10) for an uncontaminated patient classified initially as a yellow code, later on classified as a red code, is being depicted below.

Wireless compatibility with electronic management systems is accomplished by embedding NFC tags under the upper two removable copies of the patient ID on the PFC and under the printed PVC sheet of the back of the PFC on the position of the second upper removable copy of the patient ID. This way, when transferring the upper first removable copy from the PFC to the bracelet, the NFC tag is transferred as well thus providing wireless management capabilities to the bracelet itself. The same is true for the second copy in order to be transferred onto the hospital

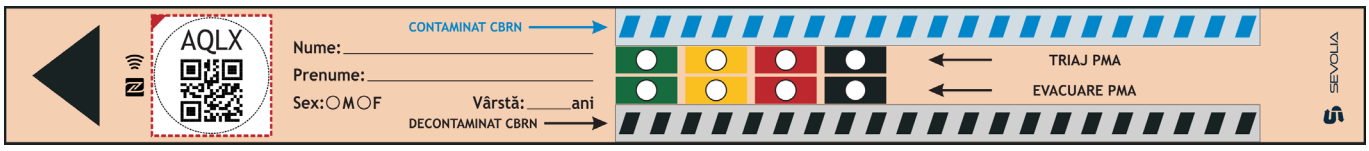


Fig. 8. The patient bracelet paired with the upgraded model (in use)

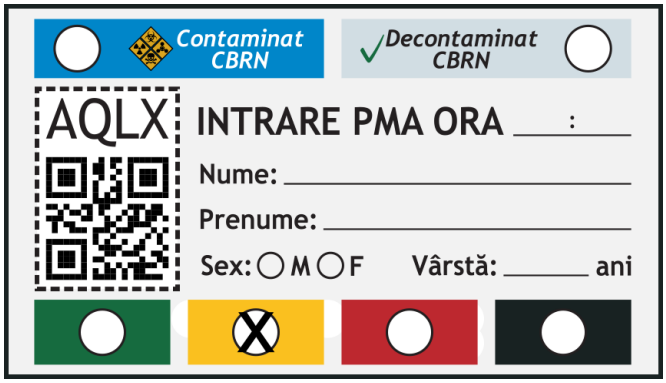


Fig. 9. The removable AMP entry point label (in use)

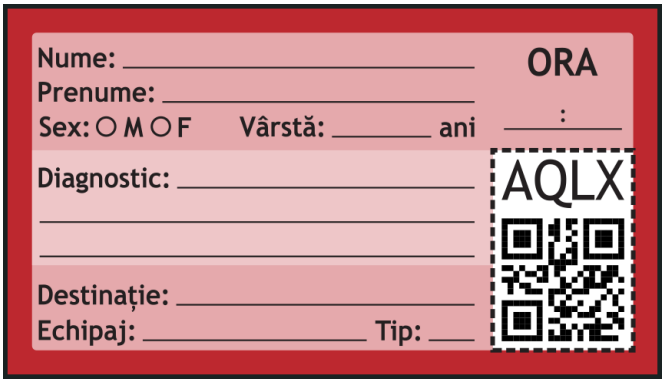


Fig. 10. The removable AMP exit point label (in use)

patient chart. Similar NFC tags may be also applied under each of the 5 copies of the patient ID on the removable tags in order to extend the wireless functionality at the entry and exit points of the AMP, using the corresponding removable labels as carriers for the tags. All NFC tags are pre-written electronically so that they will express the same ID as shown by the 4 characters and also encoded by the QR tag.

The format of the encoded information for both the NFC tags and QR tags contains proprietary validation strings and a specific data format in order to limit their use for the designed purpose and restrict any unauthorized access.

The integration of all available elements for enabling the electronic management using the PFC and patient bracelet is depicted below (Fig. 11, 12, 13).

Physical Analysis

The PFC is fabricated using a 2 mm thick 214x200 mm free-foam PVC sheet fitted with 4 10x1 mm N52 Neodymium disc magnets for locking the last 4 removable elements. A color laser printed plasticized self-adhesive 282.5x200 mm (565 cm^2) paper sheet wraps the PVC sheet and envelops 5 25x10 mm 0.5 mm thin steel plates for the removable elements.

The patient bracelet is made out of the same color laser printed plasticized self-adhesive 282.5 x27.7 mm paper sheet. The back of the left end can also be peeled off in order to provide a locking mechanism over the victim's arm or leg.

The 4-character IDs together with the corresponding QR codes are printed separately on the same laser printed material and are superimposed with the NFC tags over the designated spaces.

Content Analysis

Besides using a different strategy for selecting the severity code and contamination status there is a number of changes to the different sections of the PFC. The main differences are highlighted by layout, spatial dimensions including surface and content.

The severity code selection element on the official model is a shape formed by a half ellipse and a rectangle adding up to an area of 7.80 cm^2. There are 4 such elements on the front side and another 4 on the back side, totaling an area of 62.37 cm^2. The upgraded model uses rectangular elements of 26.32 cm^2, 4 on the front side plus another 4 on the back side, while another 4 identical areas act as severity code indicators but are used as evacuation tags. This totals 210.58 cm^2, without the entry point tag which has the same area of 26.32 cm^2. Thus, there is an increase of dedicated area of 237.62% between the upgraded model and the official model without considering the triage entry element.

Contamination status is marked over the front side on an area of 5.38 cm^2 on the official model whereas on the upgraded model the corresponding area is 26.90 cm^2, a 400.44% increase.

Patient identification by assigning character identifiers and visual elements is achieved by using 1D barcodes and numerical characters on the official model whereas these are replaced by 2D QR codes and alphabetical characters on the upgraded model. The official model has 2 such elements covering an area of 2.28 cm^2 each, while the upgraded model has 5 elements covering 2.46 cm^2 each and another 2 covering 6.16 cm^2 each. The AMP chart number of 0.62 cm^2 on the official model has been removed and no such element appears on the upgraded model. This totals 5.17 cm^2 on the official model and 24.62 cm^2 on the upgraded model, making up an increase of 376.21%.

The space and time event coordinates are split into two sections on the official model, the main coordinates being located at the top of the front side while the extraction place is located next to the legal data for patient identification. The upgraded model lacks the AMP location and all other elements are grouped together in a dedicated section. There is a total dedicated area of 7.45 cm^2 on the official model and 8.89 cm^2 on the upgraded model, which is a 19.31% increase.

Legal data for patient identification has a dedicated section on the official model covering an area of 48.41 cm^2, but only 24.99 cm2 of that – 51.63% – is being put to use for this purpose, whereas the upgraded model has a 32.64 cm^2 section, with a 30.61% increase in active space. The home address format is condensed so that it is easier to fill it in by allowing free flow input in this regard given the

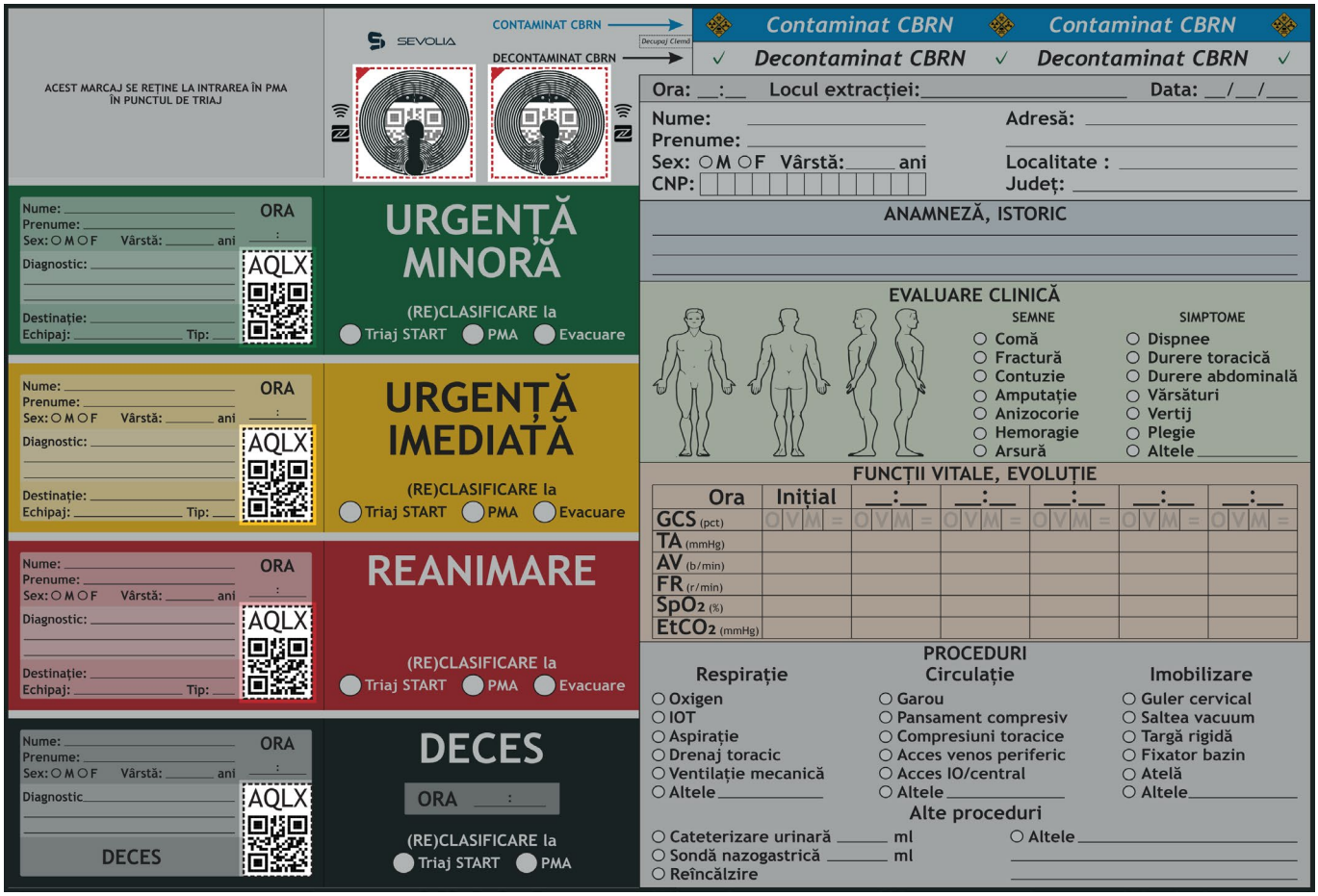


Fig. 11. Electronic management elements locations on the front side of the upgraded model

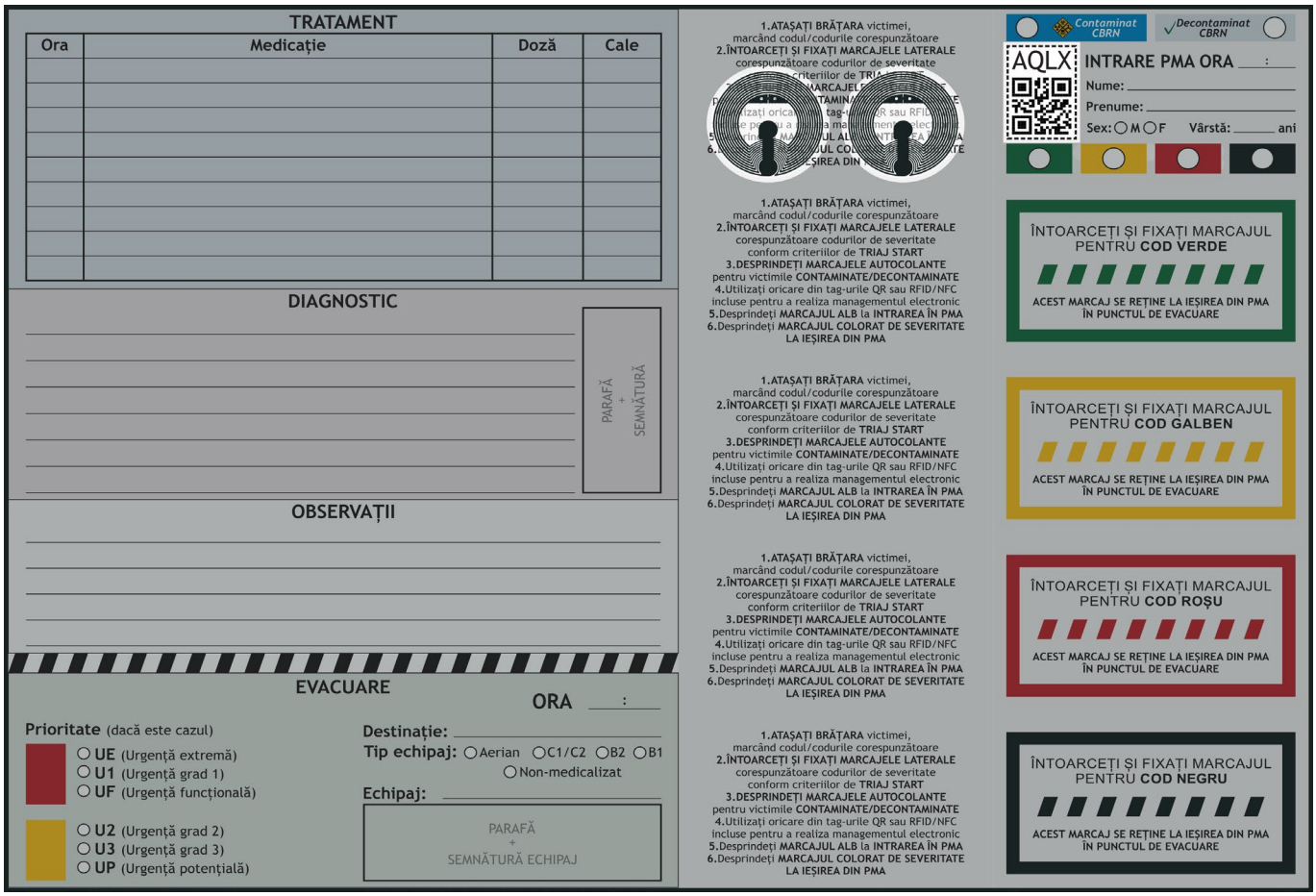


Fig. 12. Electronic management elements locations on the back side of the upgraded model

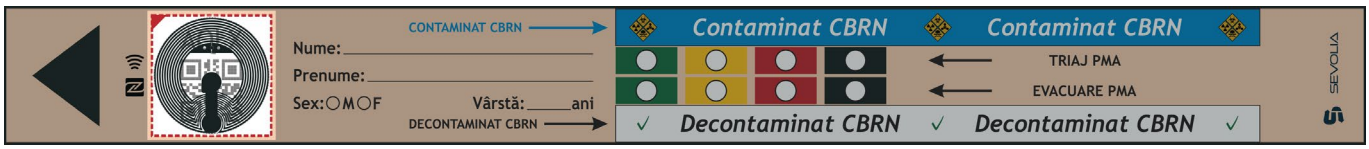


Fig. 13. Electronic management elements locations on the patient bracelet paired with the upgraded model

heterogeneity of text strings to be used while maintaining a restricted total space.

There is an exclusive patient history and event circumstances section on the upgraded model that covers an area of 26.76 cm^2.

The clinical evaluation section has two modules for each PFC model, and the diagnosis section and initial vital parameters are being added to this section as well on the official model. The first module is a schematic representation of the human silhouette and the second one contains a listing of signs and symptoms. The first module with the front and back human silhouette covers 38.10 cm^2 out of the dedicated 58.55 cm^2 area – 65.08% – on the official model, while the human silhouette is depicted with the front, back, left and right sides on the upgraded model covering 19.99 cm^2 out of the dedicated 36.91 cm^2 – 54.15%. This outputs a total decrease of 47.54% for the overall surface of the first module. The second module covers 16.55 cm^2 on the official model and 23.04 cm^2 on the upgraded model, thus increasing the dedicated area with 39.21%.

Vital signs are split into two sections on the official model, only the initial parameters being left on the front side covering 10.95 cm^2, while their evolution constitutes a separate section on the back side. The ladder covers 59.65 cm^2 out of which 5.53 cm^2 are left for an unknown parameter. The upgraded model has a single unified section of 59.34 cm^2 on the front side. Thus, there is a slight 15.96% decrease in the total area between the upgraded and the official model.

Procedures being performed cover 46.71 cm^2 on the official model but 81.40 cm^2 on the upgraded model – that is 74.26% more dedicated area. Likewise, the treatment section covers 49.93 cm^2 on the official model and 92.65 cm^2 on the upgraded model, increasing the dedicated area with 85.57%.

The diagnosis section is listed on the front side on the official model inside the clinical evaluation section and it covers an area of 23.33 cm^2. The upgraded model contains this section on the back side and includes an area for the physician's imprint and signature. The active space is 62.74 cm^2, making up an increase of 168.92%.

The area available for making observations appears as a dedicated section on the back side of the official model, also containing a separate area for listing the medical team. Active space covers 52.21 cm^2. The corresponding section, without any additions, is present on the back side of the upgraded model and covers 50.87 cm^2, which is a slight downward difference of 2.55% for the upgraded model.

The official model contains the section for listing the medical team, which covers 9.19 cm^2, but it is not adapted to an imprint shape. The upgraded model contains only a section for the physician's imprint and signature adapted to the shape of the imprint, covering 7.10 cm^2, thus decreasing the dedicated surface with 22.76%.

The evacuation section on the PFC is included on the front side of the official model and covers a total area of 32.61 cm^2. It does not incorporate any adapted area for the mobile crew's imprint and/or signature. On the upgraded model, the evacuation section is included at the bottom of the back side, clearly separated from the upper portion by a patterned separator, covering 66.46 cm^2, which is 103.78% more dedicated space than on the official model. It also includes an adapted area for the mobile crew's imprint and/or signature.

The last exclusive section containing instructions (5 identical groups) for using the PFC and patient bracelet covers 136.84 cm^2 of the back side on the upgraded model.

The evacuation note to be removed from the PFC covers 32.46 cm^2 on the front side of the official model, out of which 2.35 cm^2 are dedicated to a barcode label, and 32.48 cm^2 more on the back side, thus totaling an active area of 62.59 cm^2. The upgraded model has 4 notes available for removal differing by the background color representing the severity code at the moment of the evacuation from the AMP. Each of them covers 23.25 cm^2 of active space (an extra area of 3.11 cm^2 is dedicated to the identification visual elements) and is printed only on one side – the back side is pre-filled with instructions on how to operate the dynamic triage mechanism. For a single evacuation note there is a decrease in dedicated area of 62.85%. However, the overall area available for all 4 evacuation notes is 93.00 cm^2, upscaling the dedicated area with 48.58% in the favor of the upgraded model. Also, the upgraded model evacuation notes do not mention the initial code of severity. This is available together with the patient's identification data and contamination status on the entry point note which covers the same area of 23.25 cm^2 plus 3.11 cm^2 for the visual identification elements.

The total active surface on the official model is 507.20 cm^2, while the printed area including inert space is 551.06 cm^2. This leaves 7.96% of the actual printed area for inner dead space. The printed area with exterior dead space is 757.54 cm^2. The overall area available for printing by canvas design is 1148 cm^2. The official model does not contain any sections for instructions, the inert and dead space being identical. Thus, the total dead space by design on the official model is 55.82%.

The total active surface on the upgraded model is 914.80 cm^2, while the printed area including inert space is 1068.56 cm^2, leaving 14.39% of the actual printed area

for inert space. Since 139.51 cm^2 are used for instructions, inner dead space covers 1.33% of the actual printed area. The printed area with exterior dead space is 1130.31 cm^2, with an external dead space of 5.43%. The total dead space by design on the upgraded model is 6.72%.

The active surface increased 80.36% from 507.20 cm^2 on the official model to 914.80 cm^2 on the upgraded model ($p<0.0001$). Instructions are printed on 139.51 cm^2 only on the official model. Inner dead space dropped 67.51% from 43.86 cm^2 on the official model to 14.25 cm^2 on the upgraded model ($p=0.0002$). Outer dead space dropped 88.97% from 689.18 cm^2 on the official model to 76.00 cm^2 on the upgraded model ($p<0.0001$). The patient bracelet is not included for these computations. The physical total format of the PFC as a physical object remained unchanged – the A4 sheet.

The Patient Bracelet

The patient bracelet is made out of the same plasticized printed material as the PFC but does not include the free-foam PVC core. It has the same total length as the PFC and it is 27.7 mm wide. It is secured against the patient's arm or leg by peeling off the self adhesive left back end – marked on the front by a black arrow – and fitting that end on the opposite one.

It is designed with a dedicated area to fit the detached bundle of NFC tag and QR code together with the 4-characted ID at the left end. It accounts for patient identification data (name, sex, age), contamination status and severity code. The contamination status is set by the same peel-off mechanism, and two severity codes can be marked – at the entry and exit point of the AMP.

Discussions

The upgrade process results in almost doubling the available active area without the need to change the document size format of the product. Even more so, inner and outer dead spaces are greatly reduced while instructions are added.

Unnecessary elements like AMP chart number and the unspecified vital sign line have been removed, other elements like the expanded address format and medical team complement have been modified. Adjustments were made in unifying previously split sections like vital signs and their evolution, and displacing sections like diagnosis and evacuation in order to maintain a more laminar flow of operation.

Ten sections significantly have enlarged their designated active area while keeping the same content. Extra sections are added, like patient and event history as well as specific instructions for the user.

Visibility is greatly improved by using larger fonts given the extra reach of active space on most sections and by adding shades of different colors to highlight different sections.

Dynamic triage is made available by using side flipping elements that can be detached and later on used for inventory at the entry and exit points of the AMP. Electronic management is enabled by implementing wireless NFC tags, replacing 1D barcodes with 2D redundant QR codes and assigning 4-character ID codes to allow 439400 different combinations. This amount can be largely augmented by switching from Latin alphabet restricted characters to alphanumeric characters, thus expanding the scale to 1632960 different combinations while keeping the 4-character ID format. The previous range of possible combinations when using the 1D barcode is unknown.

Electronic data security features are in place by using proprietary format strings and data formats to restrict access to and from the electronic management elements.

The user benefits from solid weatherproof and weather resistant materials in an easy to handle format, allowing the product to be easily attached to the patient or different nearby objects and easily removed in order to continue the flow of operation.

The patient bracelet allows for an easy marking of the corresponding patient, preserving only the most important features for this purpose i.e. identification elements including the ones for electronic management, contamination status, and AMP entry and exit points severity codes.

Conclusions

The upgraded model offers a variety of improvements for both the overall rescue effort as well as the end user of the product. It allows for previously unavailable features like unlimited dynamic triage and enables the use of electronic management solutions.

Pre-printed identification codes make it very easy to safely operate a very large number of products and seamlessly identify them by visual or electronic means, without the need to dedicate time and effort for these actions, thus decreasing the chance for error.

The text strings of the content can be easily modified to accommodate internationalization.

The downside of the end product is its significantly higher cost in comparison to a regular A4 paper sheet. However, if the official model is printed on weatherproof and weather resistant materials, the corresponding costs get in a significantly closer range. Agencies may also decide on wireless technology and the number of wireless tags to be used – or by not including any at all –, largely adjusting the total cost in both directions. Some of the costs may be lowered by recycling the neutral composing elements like the free-foam PVC sheet, magnetic and metallic elements, but this may set the need for a recycling service that would have a cost of its own.

The variety of options and features makes the product fit for adoption at different scales, either local, regional, national or even international. Its implementation requires training and a learning curve.

Given the fact that the Romanian official model came into play relatively recent and its adoption is not of the clearest, it is hard to imagine that switching to any up-

graded model would be feasible despite of the numerous advantages.

However, carrying out usability studies using professionals to validate the proposed concept is desirable and may open the door for a future adoption as well as performing adjustments and optimizing the product for the version.

Conflict of interest

None to declare

Authors' contribution

AS – Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, visualization, writing original draft
SMC – Data curation, supervision, validation
CMB – Data curation, supervision, validation

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RESEARCH ARTICLE

Ultrasonography: New Insights in its Applicability to Explore Muscle Mass and Musculoskeletal Inflammation in Critically ill Patients

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Objective: The main aim of the study was to explore muscle mass changes and to investigate musculoskeletal inflammation in critically ill patients. **Methods**: A pure observational study that comprised two musculoskeletal analyses was conducted. Ultrasonography was used to determine the inflammatory process and muscle mass modifications. We assessed the presence of musculoskeletal inflammation and muscles area reduction. We recruited 26 patients and we performed both imaging investigations (shoulder and hip joints, biceps brachii and rectus femoris areas) and anthropometric measurements (mid-upper arm circumference). **Results**: More than 70% of patients were classified with low muscle mass, over one half of sarcopenic patients being over-weight and 17% being obese. The relationship between the length of stay in intensive care unit, mechanical ventilation and presence of low mid-upper arm circumference, highlighted a significant difference when comparing sarcopenic and non-sarcopenic groups. Musculoskeletal inflammation expressed by step-down lesions, calcifications and osteophytes, is common in these patients. Statistically significant results were obtained when comparing the dimensions of the investigated muscles. Good inter-observer variability in day 3 of assessment for biceps brachii and rectus femoris was noticed. **Conclusions**: More than 1/3 of critically ill patients included in the present study was classified with low muscle mass. The length of stay in intensive care unit and the length of mechanical ventilation had an important impact on sarcopenic patients. Musculoskeletal impairment was frequent, reflected by presence of enthesitis lesions in joints and by dynamic reduction of muscle area.

Keywords: ultrasonography, low muscle mass, sarcopenia, critically ill patients

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Introduction

Various concepts have been proposed in order to define critical illness, both acute and chronic. There is not a precise mark of differentiation between acute critical illness and chronic critical illness, the evolution from acute to chronic being progressive [1, 2]. Considering that critically ill patients are distinguished by the needs of special care and large numbers of intensive care unit (ICU) resources, their management should comprise not only the life support treatments, but also the prevention of the short and long-term negative sequela [3]. Taking into account the frailty syndrome and disability that characterize critically ill patients, the assessment of other comorbidities should be made.

Sarcopenia, muscle mass impairment and musculoskeletal inflammation as pre-existing comorbid diseases are vehement predictors of life quality in post-ICU patients [4]. Designed as a major public health issue, the highest prevalence estimation shows a 63.8% increase from 2016 to 2045 (from 19.740.527 to 32.338.990 persons) [5]. The European Working Group on Sarcopenia in Older People (EWGSOP) enclosed in the diagnostic criteria of sarcopenia the presence of both low muscle mass and low muscle function (strength and performance) [6]. There are many tools to assess sarcopenia (muscle mass: computed tomography, magnetic resonance imaging, dual energy X-ray absorptiometry, bioimpedance analysis; muscle strength: handgrip strength, knee flexion/extension; physical performance: short physical performance battery, usual gait speed, stair climb power test) [6], but there are few data regarding the use of ultrasonography and its applicability to explore muscle mass and musculoskeletal inflammation in critically ill patients.

The main purpose of this study was to explore the muscle mass changes and to investigate the musculoskeletal inflammation in critically ill patients. It is important to recognise muscle impairment by repeating dynamically the same measurements in the same patients, in order to find out the ubiquity of sarcopenia in critically ill patients and to optimize the diagnostic.

Material and method

We conducted a pure observational, non-interventional study that comprised two musculoskeletal analyses.

On the one hand, we assessed the presence of musculoskeletal inflammation in critically ill patients. As ultrasonography is considered a valuable tool to determine the inflammatory process, some of the joints' pathological features were examined in the interest of detection of en-

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thesitis. For 15 critically ill patients both scapulo-humeral and coxo-femoral joints were observed, by looking for changes in muscular inflammation: bursitis, osteophytes, entesophits, calcifications, erosions, Doppler signal [7], by an experienced operator.

On the other hand, a group of 11 critically ill patients followed three ultrasonographic determinations on the biceps brachii and recuts femoris area, corresponding to 5 days period (day 1, day 3, day 5), in order to identify muscle area reduction. These tests were completed by two operators (novice and experienced) in the furtherance of inter-variability assessment.

Anthropometric measures have been used to estimate muscle mass. Calculations based on mid-upper arm circumference (MAC) and skin fold thickness were adopted. Applying the following formula: *MAC= mid-upper arm circumference – (3.14*skin fold thickness)*, low muscle mass was classified with MAC <21.1cm for men and MAC <19.2cm for women [8].

We completed a data base where the following information were noted: demographic data, reasons of admission in the ICU, anthropometric measurements, results of imaging investigations, length of stay in ICU, necessity of mechanical ventilation (MV), severity scores: GCS (Glasgow Coma Scale), APACHE II (Acute Physiology and Chronic Health Assessment), SOFA (Sequential Organ Failure Assessment) and secondary effects of intensive care hospitalisation (metabolic, protein, renal dysfunctions).

To operate statistical analysis Microsoft Office Excel package, GraphPad Prism 6 and SPSS were used. To verify Gaussian distribution Kolmogorov-Smirnov test was used and a $p \leq 0.05$ was set as reference, with a confidence interval (CI) of 95%. Intraclass correlation coefficient (ICC) was used to attest inter-variability between two operators.

Prior to the onset, the approval from the Ethics Committee of Targu Mures Clinical County Emergency Hospital was obtained. All data used have no personal character and conformed to the Helsinki Declaration.

Results and Discussions

Twenty six critically ill patients (62% men, 38% female) that met inclusion criteria (critically ill patients admitted to the ICU for a stay of at least 24 hours; patients over 18 years old; patients for whom imaging investigations and anthropometric measurements have been performed) were included in the study.

Type of admission was acute medical for 65% patients and acute surgical for 35% patients. The most common cause of admission was respiratory failure (31%), followed by neurologic degradation (21%), after-surgery life support necessity (14%) and sepsis (11%). 85% of these patients presented pre-existing comorbidities, nearly one third being diagnosed with diabetes. With a high prevalence of diabetes worldwide, this result confirmed Bianchi's hypothesis that diabetes was a risk factor contributing to several complications including muscle frailty and sarcopenia [9].

Taking into consideration demographic data and anthropometric measurements, two groups of conditions were formed: sarcopenic group (muscle mass (MAC) under the cut-off) and non-sarcopenic group (muscle mass (MAC) above the cut-off). More than 1/3 of patients were classified with low muscle mass by following the method indicated in Gariballa's article published in Clinical Nutrition. These groups were homogenous with no significant difference regarding the age (average age 62.77±15.17 years old) or body mass index (BMI) (Table I.) Despite of this, the study pointed out that 53% of sarcopenic patients are over-weight and 17% are obese. Sarcopenic obesity was described by Baumgartner as low muscle mass index less than 2 SD below the sex-specific reference for a young, healthy population [10]. Besides diabetes, sarcopenic obesity should be considered a risk factor for critically ill patients, acting synergistically to develop multiple negative outcomes by increasing morbidity and mortality [11].

We assessed the short term impact of sarcopenia in critically ill patients. We identified a significant difference between sarcopenic and non-sarcopenic patients when we considered length of stay (LOS), MV and low MAC. (Table II.)

Severity scores based on laboratory results and clinical data (GCS, APACHE II, SOFA) were noted for each patient, but there was no significant difference between sarcopenic and non-sarcopenic patients ($p>0.05$). (Table III)

Regarding musculoskeletal inflammation, the most common lesions observed both at scapulo-humeral and coxo-femoral joints were step-down lesions (28% shoulder, 25% hip), calcifications (22% shoulder, 35% hip), and osteophytes (24% shoulder, 28% hip) (Figure 1, Figure 2). As consequence, musculoskeletal inflammation could be a pre-existing comorbidy or the critical care environment and immobility could predispose to muscle impairment.

Table I. Patients' characteristics (sarcopenic versus non-sarcopenic)

	p
Age	0.069
BMI	0.281

Age: patients >18 years old were included; BMI: weight/ (height x height), considering BMI >25.00 overweight; BMI >30.00 obesity

Table II. Short term impact of low muscle mass in critically ill patients

	p	R squared	CI (95%)
Length of stay	0.006	0.269	3.452 – 19.130
MV (hours)	0.003	0.013	-6.167-3.475

Table III. Severity scores in sarcopenic versus non-sarcopenic patients

	p	R squared	CI (95%)
GCS	0.569	0.013	-6.167- 3.475
APACHE II	0.267		
SOFA	0.430	0.026	-9.885- 4.351

In order to investigate rapid reductions in muscle mass, two operators consecutively checked every patient by using ultrasound diminution in the area of biceps brachii (BB) and rectus femoris (RF) in day 1, day 3 and day 5 as presented in Table IV. and Table V.

Both observer 1 and observer 2 obtained statistically significant results when comparing the dimensions of the investigated muscles. There was a statistically significant decrease in muscle mass at BB level between the first two evaluations and the third assessment (p = 0.032, r = 0.0380, CI = -0.612-0.032; p = 0.033, r = 0.379, -0.523- -0.027). Also, there was a decrease in the ultrasound measured area at the left BB between the second and third investigations (p = 0.037, r = 0.474). At the level of the lower limbs, both observer 1 and observer 2 surprised a diminished area measured at the left RF level between evaluation 1 and assessment 2 (p = 0.038, r = 0.370, CI = -0.568-0.018; p = 0.001, r = 0.690, CI = -0.610 -0.208). These results sustain Nakanishi's observations that skeletal muscle weakness is common in critically ill patients. The letter published in Intensive Care Med (2018) showed atrophy in muscle of critically ill patients in both upper and lower limbs.

The secondary objective was to evaluate inter-observer variability of the results, analysing the same subjects. It has been pointed out in the literature that a kappa value of between 0.7-0.9 gives confidence in results [13]. In the current study, Intraclass Correlation Coefficient (ICC) proves good inter-observer variability at the following levels: BB right assessment 3, RF left assessment 3, RF right evaluation 1 (Table VI.). (Insert table VI). These results demonstrate that more practice improves the quality of the investigation.

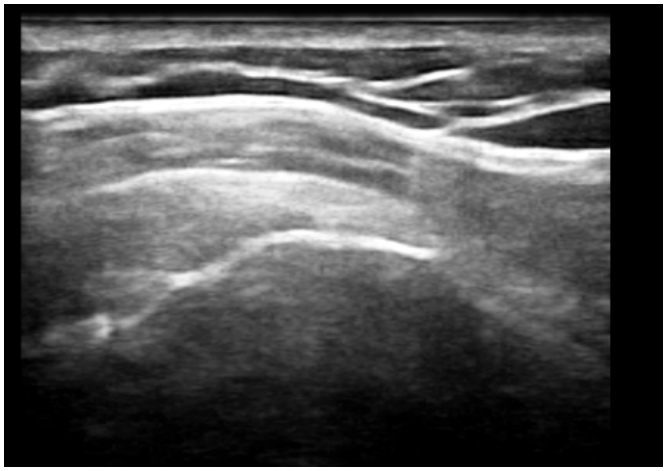

Fig. 1. Osteophytes and erosions shoulder joint

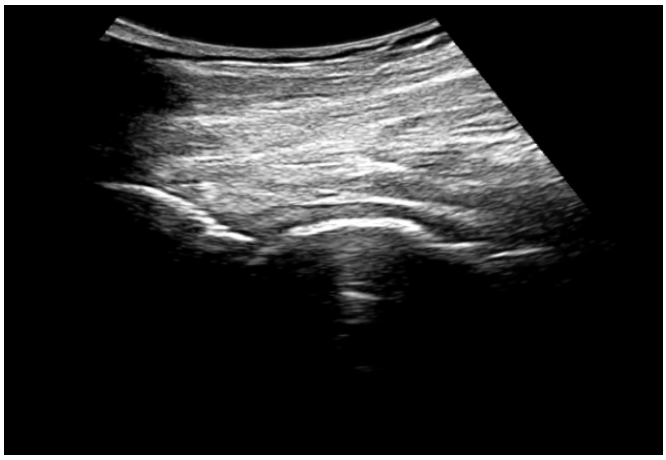

Fig. 2. Calcifications and erosions hip joint

Table IV. Dynamic changes in muscle mass (observer 1)

	p	R	CI (95%)
BB right ev 1/2	0,705	0,014	-0,317- 0,223
BB right ev 1/3	0,032	0,380	-0,612- -0,032
BB right ev 2/3	0,033	0,379	-0,523- -0,027
BB left ev 1/2	0,564	0,038	-0,381- 0,655
BB left ev 1/3	0,695	-0,407	
BB left ev 2/3	0,037	0,474	
RF right ev 1/2	0,492	0,340	
RF right ev 1/3	0,105	0,291	
RF right ev 2/3	0,058	0,731	-0,541- 0,011
RF left ev 1/2	0,038	0,370	-0,568- -0,018
RF left ev 1/3	0,131	0,356	-0,443- 0,066
RF left ev 2/3	0,261	0,446	-0,091- 0,302

Ev 1/2, 1/3, 2/3 refers to the comparation between muscle mass assessments day 1 / day 2/ day 3

Conclusions

Even if more than half of the critically ill patients were obese at admission, they were classified with low muscle mass. Results sustained that the LOS in ICU and the length of MV negatively impact sarcopenic patients. Musculoskeletal impairment is frequent as pre-existing condition or due to ICU immobility, reflected by presence of enthesitis lesions in joints and by dynamic reduction of muscle area both in upper and lower limbs.

Authors' contribution

OEB – Conceptualization, data curation, formal analysis, investigation, methodology, writing original draft
ARJ – Formal analysis, investigation, methodology

Table V. Dynamic changes in muscle mass (observer 2)

	p	r	CI (95%)
BB right ev 1/2	0,885	0,024	-0,346- 0,395
BB right ev 1/3	0,240	0,123	
BB right ev 2/3	0,097	0,388	
BB left ev 1/2	0,999	0,224	
BB left ev 1/3	0,425	0,307	
BB left ev 2/3	0,064	0,620	
RF right ev 1/2	0,556	0,413	
RF right ev 1/3	0,112	0,154	-0,726- 0,090
RF right ev 2/3	0,625	0,248	
RF left ev 1/2	0,0087	0,247	-0,698- 0,130
RF left ev 1/3	0,0011	0,690	-0,610- 0,208
RF left ev 2/3	0,951	0,622	-0,189- 0,200

Table VI. Inter-observer variability assessment

	Significance	ICC	CI (95%)
RF right ev 1	0.005	0.804	0.342-0.946
BB right ev 3	0.041	0.714	-0.137-0.929
RF left ev 3	0.041	0.701	-0.151-0.920

RGB – Formal analysis, investigation, methodology
SMC – Conceptualization, methodology, supervision, visualization
MC – Conceptualization, methodology, supervision, validation, visualization

Conflict of interest

None to declare.

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RESEARCH ARTICLE

New Insights in Growth Hormone Stimulation Tests Protocols

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Objective: The objective of this study was to analyze the performance of 2 stimulation tests used in the diagnosis of growth hormone deficiency. **Method**: A retrospective study was conducted on a non-random sample of 310 patients, between 2 and 20 years old, who were hospitalized in the Mureș County Hospital's Endocrinology Department and in the National Institute of Endocrinology C.I. Parhon with short stature between 2009-2015. Inclusion criteria: all subjects who underwent growth hormone stimulation tests in accordance with the national protocol. Microsoft Office Excel was used for data collection and MedCalc v 12.5 was used for statistical analysis. **Results**: From the total of 310 patients, 102 were diagnosed in Târgu Mureș and 208 in Bucharest. Sex ratio favored boys (boys:girls 1.64:1). In 173 subjects growth hormone deficiency was confirmed. For both tests the percentage of maximum response was the highest for the 60 minutes blood sample regardless if the test were positive or not. Both tests have 100% sensitivity and negative predictive value, with the highest specificity for the 60 minutes clonidine and 30 minutes insulin. The false positive rate was 60% for the insulin test and 27.2% for clonidine for Târgu Mureș sample and 86.9% for the insulin test and 62.5% for clonidine for Bucharest sample. The concordance of the 2 tests was 49.36%. **Conclusions**: Stimulating growth hormone testing has a number of limitations but is still needed in some auxological circumstances. We recommend performing the clonidine test first to exclude idiopathic short stature and then the insulin tolerance test for the diagnosis of growth hormone deficiency.

Keywords: stimulation tests, growth hormone deficiency, auxology

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Introduction

The growth and development of the child is under the strict control of the endocrine system. Since childhood, an important role is played by growth hormone-releasing hormone/ growth hormone/ insulin-like growth factor 1 (GHRH / GH / IGF1) and thyroid hormones, whereas during puberty the main role is played by sex steroid hormones [1,2]. The primary function of GH is to promote growth, being achieved mainly through IGF1. [3].

A child who is under 2.5 standard deviation (SD) for age, sex and population average height, will be classified as growth delay, while a child whose measurements exceed 2.5 SD statistically calculated average, will be diagnosed with tall stature [1,2,6,7]. In childhood, the diagnosis of growth hormone deficiency should be based on auxology, which will allow the comparison of the individual's growth model with the reference values from the growth charts [3,9-11]. In order to complete and confirm the diagnosis, static laboratory tests will be performed such as GH, IGF1, IGFBP or dynamic tests like GH stimulation tests [3,6,12]. In all cases of confirmed GH deficiency, it is mandatory to perform a magnetic resonance imaging of the pituitary gland in order to diagnose all structural anomalies associated with pituitary dysfunction [6].

Patients who will undergo GH-IGF-1-IGFBP axis evaluation belong to one of the following categories: (1) severe short stature (height <-3 DS); (2) height <-1.5 DS below the target genetic height; (3) medium stature deficit (height between -2 and -3 DS) and growth deceleration (growth velocity <-1 DS for 2 years or <-2 DS for 1 year); (4) history of brain tumor, cranial irradiation or other pituitary pathology; (5) imaging evidence of pituitary pathology [2,3,6].

Although the routine use of GH-stimulation tests is highly controversial [12,15], currently they are included in the diagnostic protocol of GH deficiency [3,6,7,10]. Stimulation tests are classified into two categories: screening tests, which include GH value after intense physical exercise, food deprivation, levodopa or clonidine stimulation, and definitive tests which include stimulation of GH secretion with insulin, arginine or glucagon [3,7]. To increase the specificity of these tests, the results of at least two of them are usually combined [3,7,10,16].

In order to establish a positive diagnosis of pituitary dwarfism, the national protocol requires the following assertions: the height deficit must be minimum 2.5 DS; stature deficit between - 2 and - 2.5 DS and the growth velocity a year before 2 DS below the average velocity for age and sex, or growth velocity in the year before at least 1.5 DS below the average for sex and age; in children with post-irradiation or postoperative acquired GH deficiency, increase in stature deficit by 0.5 DS per year; bone age should be over 2 years late; the child (generally over 3 years) must have 2 negative GH stimulation tests or 1 negative test and an IGF I value in serum below the lower

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limit of normal for age; in pubertal patients to exclude the transient GH deficiency, priming with sex steroids can be performed prior to dynamic GH testing[17].

Short stature diagnosis and treatment are quite controversial within the worldwide pediatric endocrinologists practice. Growth deficiency has a steady increase in prevalence, currently representing at least 2.5% of pediatric pathology in 3 to 16-year-old infant population. Currently, the diagnosis and treatment of growth hormone deficiency is possible in 6 centers in our country, but with some limitations and differences in GH stimulation testing.

The aim of this study was to analyze the performance of two growth hormone stimulation tests (clonidine and insulin test), by assessing the results obtained in two centers in Romania, in Târgu Mureş at the Endocrinology Clinic and in Bucharest at the National Institute of Endocrinology CI Parhon in order to optimize as much as possible the diagnostic and therapeutic protocol of this pathology.

Method

We conducted a cross-sectional study on a group of 310 patients with a suspicion of GH deficiency, aged between 2 and 20 years, admitted to the Endocrinology Clinic of Târgu Mureş and the National Institute of Endocrinology CI Parhon Bucharest, between January 2009 and October 2015.

A written informed consent was obtained for each patient included in our study. All data that was used has no personal character and it was performed according to the Helsinki Declaration. Prior to the inclusion of the patients in this study the approval from the head of both endocrinology departaments, Târgu Mureș and Bucharest, was obtained.

All children suspected with growth hormone deficiency ((1) severe short stature (height <-3 DS); (2) height <-1.5 DS below the target genetic height; (3) medium stature deficit (height between -2 and -3 DS) and growth deceleration (growth velocity <-1 DS for 2 years or <-2 DS for 1 year); (4) history of brain tumor, cranial irradiation or other pituitary pathology; (5) imaging evidence of pituitary pathology), for whom GH-stimulation tests were performed according to the national protocol, were included. Patients who presented certain genetic syndromes such as: Turner syndrome, Prader Willi, Bardet Biedl, or patients who have been small for gestational age were excluded from our study. According to the current protocol, GH deficiency is defined as GH value of <10 ng / ml during 2 GH stimulation tests (for each blood sample obtained). If IGF1 is lower than the lower limit of normal, a single dynamic test is performed.

The test protocols differ between the two centers, especially regarding blood sampling times. Therefore, in The Endocrinology Clinic from Târgu Mureş, for clonidine stimulation test, blood samples are collected to obtain a basal GH value, then the pharmacological agent is administered (Clonidine 5 mcg/kg, sau 150 mg/m^2, maxim 250 mcg) followed by sampling at 30, 60, 90 and 120 minutes. In the case of the CI Parhon Institute, the post-administration of clonidine (same dosages) blood sampling is done at 60 and 120 minutes.

For the insulin tolerance test, in Targu Mures, is used the same protocol as for clonidine, thereby blood samples are collected for basal GH monitoring and then at 30, 60, 90, 120 minutes after the administration of insulin 0.05-0.1 UI/kg i.v. în bolus. In Bucharest, after insulin (same dosage) administration sampling is done at 30 and 60 minutes.

In order to take into account the dosage of growth hormone obtained from insulin stimulation, the glucose level after insulin administration must fall to half the blood glucose fasting level or below 2.2 mmol/ l (40 mg / dl).

Data was collected using Microsoft Office Excel package, and graph pad prism was used for statistical analysis. Discrete quantitative and qualitative binary variables were used. For comparison of medians, the Mann-Whitney test was performed, chi square test for the performance of diagnostic tests and Spearman coefficient for correlation, with a significance threshold of alpha = 0.05.

Results

Of the total of 310 patients, 102 children were diagnosed in Târgu Mures and 208 in Bucharest. Gender ratio favored boys 1.64:1, accounting for a total of 193 male and 117 female patients.

Average age for Târgu Mureş group was 9 years ± 3.8 years (8.8 for girls and 9.2 for boys), of whom 59 were less than 10 years old and 43 had exceeded the average prepubertal threshold. Bone age, analyzed for each patient according to hand radiography, ranged from 1 to 15 years, the mean difference between the bone age and chronological age for the first group was 2 years.

For the Bucharest patients, the average age was 9.7 years ± 4.1 years (9.6 for girls and 9.7 for boys), 129 of them were younger than 10 years and 79 were above this age. Bone age ranged from 1 to 17.5 years with a mean difference between this and the chronological age of 3.4 years.

We compared the results obtained during the clonidine stimulation and insulin stimulation tests for each group.

For Târgu Mureş group, we had 99 patients who underwent clonidine stimulation test and 26 for whom ITT was performed. Out of the whole group, only 23 patients underwent both stimulation tests.

We noted that for the patients from Târgu Mureş the percentage of cases who had a maximum response during the stimulation tests, was the highest for the blood sample taken at 60 minutes after the administration of the pharmacological agent. More precisely for 53.53% of children we obtained the highest GH value at 60 minutes after clonidine stimulation and, at the same time of sampling, we obtained maximum GH values for 34.61% of children who underwent insulin test (Figure 1).

For the Parhon Institute group, from the 208 investigated children, 173 were stimulated with clonidine and

85 with insulin. Only 56 patients underwent both GH stimulation tests. Thus, in Figure 2 we can observe for the clonidine stimulation test, 72.25% of the patients had a maximum response at 60 minutes after the pharmacological agent administration, while 27.75% had a positive response at 120 minutes. Blood samples collected for the insulin test, pointed out that 50.58% of children had a maximum response at 60 minutes after insulin administration, and the rest of 49.42% at 30 minutes (Figure 2).

Of the whole group, for 79 subjects, both stimulation tests were performed, 39 (49.36%) were concordant, with a gender ratio favoring boys (1.4:1).Regarding the non-concordant tests, for most cases, insulin testing revealed growth hormone deficiency, while the clonidine test revealed adequate stimulation of GH (80%). The kappa index of agreement was -0.103 (95%CI -0.261-0.055, p=0.220) (Figure 3).

For both samples the performance parameters of the two tests were calculated, against the GH deficiency definition of a maximum GH value below 10ng/ml during stimulation tests. For both centers the tests have sensitivity and negative predictive value of 100%. Specificity and positive predictive value were calculated for each test separately and for each group of patients, since the test protocol differs in these two hospitals (Table I).

Also, false positive rate was calculated for each sampling moment, and for each test as a whole, by analyzing the subjects who underwent both tests. For Târgu Mureş group, clonidine at 30 minutes and insulin at 120 minutes have the highest false positive rate. In other words, if samples were taken only at these times, most of the subjects would be considered to have growth hormone deficiency when they actually had adequate stimulation at other sampling moment.

For Bucharest group, the maximum false positive rate was at 120 minutes for clonidine and 60 minutes for insulin. The false positive rates of both tests as a whole, are higher than for Târgu Mureş center, where the clonidine test has a false-positive rate of 27.27% (Table II). We calculated the number of cases that would be misdiagnosed as GH deficiency assessing how many cases were stimulated at values above 10 ng / ml only at a certain time of sampling.

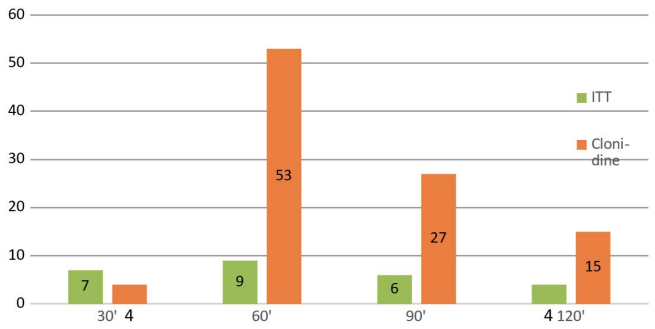

Fig. 1. Subdivision of subjects according to the maximum response of GH to the 2 tests (Târgu Mureş)

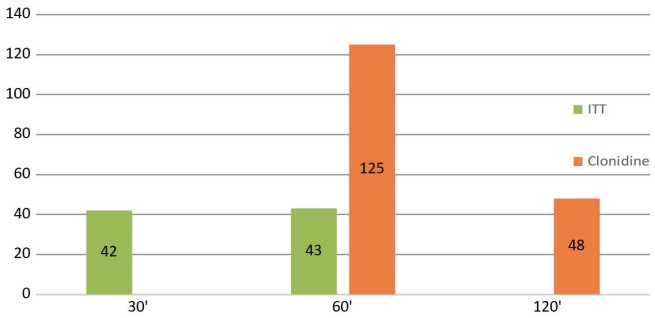

Fig. 2. Subdivision of subjects according to the maximum response of GH to the 2 tests (Buchaest)

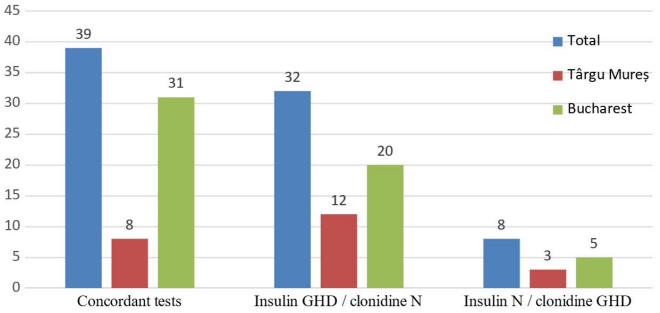

Fig. 3. The concordance of the 2 diagnostic tests

We analyzed the influence of various parameters taken into account, on the maximum response to stimulation tests. Maximum response to clonidine correlates positively with the standard deviation score for height, IGF1 and

Table I. Performance of the 2 tests

Center	Moment	Specificity	CI95%	PPV	CI95%	P
Târgu Mureş	Clonidine 30 minutes	6.82%	1.43-18.46%	57.29%	46.78-67.34%	<0.08
	Clonidine 60 minutes	86.36%	72.66-94.82%	90.16%	79.81-96.31%	<0.0001
	Clonidine 90 minutes	61.36%	45.44-75.65%	76.39%	64.9-85.63%	<0.0001
	Clonidine 120 minutes	25%	13.19-40.29%	62.5%	51.56-72.56%	<0.0001
	Insulin 30 minutes	75%	19.42-99.37%	95.83%	78.86-99.89%	0.0014
	Insulin 60 minutes	50%	6.76-93.24%	92%	73.96-99.02%	0.0171
	Insulin 90 minutes	50%	6.76-93.24%	92%	73.96-99.02%	0.0171
	Insulin 120 minutes	25%	0.63-80.58%	88.46%	69.86-97.55%	0.1481
Bucharest	Clonidine 60 minutes	89.1%	77.7-95.9	95.16	89.7-98.2	<0.0001
	Clonidine 120 minutes	18.2%	9.1-30.9	72.4	64.9-79.1	<0.0001
	Insulin 30 minutes	68.4%	43.4-87.4	92.3	84.01-97.1	<0.0001
	Insulin 60 minutes	42.1	20.2-66.1	85.7	75.9-92.6	<0.0001

negative with BMI. Maximum insulin response correlates positively with age, standard deviation score for height, and IGF1. Bone age does not correlate with the response to any of the stimulation tests (Table III).

Discussions

By analyzing the performance of the two diagnostic tests, we found that both tests had sensitivity and negative predictive value of 100%, due to the fact that the current national protocol imposes a clear cut-off for GH of 10 ng/ml. Thus we observed that in the case of Târgu Mureş, for clonidine stimulation test, the best specificity was obtained at 60 and 90 minutes blood sampling.

For Parhon Institute group, the specificity of the 60 minutes sample did not differ significantly from the Târgu Mures group, while for the 120 minutes sampling, it was significantly lower. Actually, it is worth mentioning that in case of Târgu Mureş center, for clonidine test, blood sampling at 90 minutes after the administration of the pharmacological agent, helps in diagnosing a relatively large number of children, while the blood sample taken at 30 minutes does not provide any new cases that are not further stimulated at 60 minutes. Therefore, if the elimination of the 90-minute blood sampling from the testing method can lead to over-treatment of children with growth hormone, possibly due to incomplete diagnosis, the 30 minutes blood sampling could be safely ruled out with no risk of misdiagnosing these children.

In the case of ITT, for the Târgu Mureş sample, we obtained the highest specificity for the sample taken at 30 minutes and the lowest at 120 minutes. For the Bucharest sample, the specificity of the 30-minute sample was superior to the 60 minutes one. It is not to be neglected that in Târgu Mureş clinic, the 90-minute blood sample after insulin administration diagnosed the same number of children as the 30-minute one, showing the importance of at least 3 blood samples for each test.

Test performance of these two tests has been analyzed in many other clinical trials. One of these studies reports a specificity of 79% for ITT in comparison to 85% for clonidine [22]. Another interesting aspect is that it has been shown that the specificity of the tests would improve if a lower cut-off value was used [12].

All investigations made on the whole studied group, both in Târgu Mureş and Bucharest have shown that GH deficiency is more commonly diagnosed among male patients.

The average age of diagnosis for the entire group was very close to the onset of puberty, highlighting the fact that in Romania the GH deficit is diagnosed late. Patients should be diagnosed before the age of 5 years, because the treatment results are closely related to the age when the treatment is initiated. The studies recommend to start the treatment at an early age in the prepubertal stage, the most relevant results being noticed in children younger than 4 years [6,24]. It is most reasonable to initiate postnatal growth hormone treatment when growth deficiency begins to be visible independently of the age. Therefore, favorable results have been obtained even at the age of 9 months [25].

The diagnostic tests were concordant in less than half of the analyzed results. The large number of non-concordant tests reveals that the diagnosis of pituitary dwarfism should not be based predominantly on GH stimulation test. Analyzing the correlations between anthropometric parameters and stimulation tests, we obtained statistically significant results for the clonidine test in the case of DS for BMI and IGF1. There are studies that have shown a relationship between weight, BMI and GHD. Therefore, compar-

Table II. Fals positive rate

Târgu Mureș sample		**Bucharest sample**	
Moment	Fals positive rate	Moment	Fals positive rate
Clonidine 30 minutes	93.18%	Clonidin 60 minutes	10.91%
Clonidine 60 minutes	13.63%	Clonidin 120 minutes	81.81%%
Clonidine 90 minutes	38.63%	Insulin 30 minutes	31.57%
Clonidine 120 minutes	75%	Insulin 60 minutes	57.9%
Insulin 30 minutes	25%	Insulin tolerance test	86.9%
Insulin 60 minutes	50%	Clonidin test	62.5%
Insulin 90 minutes	50%		
Insulin 120 minutes	75%		
Insulin tolerance test	60%		
Clonidin test	27.27%		

Table III. Correlation of anthropometric parameters with the maximum response to stimulation tests

	Maximum response to clonidine			**Maximum response to insulin**		
Parametre	R	95%CI	p	R	95%CI	p
SD height	0.1717	0.052-0.2865	0.0052	0.2897	0.1099-0.4509	0.0020
BA	0.1049	-0.0271-0.2334	0.1190	0.1025	-0.1011-0.2979	0.3230
DS IGF 1	0.2108	0.0886-0.3268	0.0008	0.4455	0.2871-0.5801	<0.0001
Age	-0.0598	-0.1775-0.0595	0.3257	0.4655	0.3102-0.5967	<0.0001
SD BMI	-0.1781	-0.2928-(-0.0584)	0.0038	-0.0862	-0.2644-0.0975	0.3571

Legend: SD- standard deviation; BA- Bone age; BMI- body mass index; CI- confidence interval; IGF 1- Insulin-like Growth Factor 1

ing the results of a group of children diagnosed with GH deficiency and one with an idiopathic small stature, it was showed that the BMI mean for the GH deficiency group was higher than for those in the idiopathic small stature group [26]. Another study asserts that the maximum GH value obtained from stimulation tests decreases with the increase in DS for BMI. Children who associate obesity also have a low value of endogenous GH secretion compared to normoponderal children [27].

In Romania, there are no further studies to analyze the clear differences between the protocols used for the GH stimulation tests in other reference centers.

Our proposals regarding the national protocol for GH deficiency diagnosis would be: minimum 3 blood samples for both diagnostic tests studied in order to avoid the incorrect diagnosis of this pathology or over-treatment whit growth hormone; for the clonidine test the most specific and reliable results are at 0, 60, 90 and 120 minutes, whereas for ITT is recommended to assess the 0, 30, 60, 90 minutes blood samples.

The limitations of the study were: the use of different protocols in the centers included in our study, the performance of the stimulation tests were calculated separately for each group, the results being practically difficult to compare; in ITT, there were cases included in the study group, for which the blood glucose level after insulin administration did not fall below 40 mg/dl or below half the baseline blood glucose; and the lack of data that could influence the diagnosis of GH deficiency, such as: social status, possible renal, cardiac or malabsorption disease.

Conclusions

Our study, revealed a low specificity for insulin tolerance test. During the clonidine stimulation test, the maximum specificity was obtained at 60-minutes, while in case of the insulin stimulation test, it was obtained at 30 minutes. Only half of the cases presented concordance between the two tests. BMI and DS for IFG1 significantly influence clonidine test results, whereas DS for IGF1 and age influence ITT results.

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Authors'contribution

IA – Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, writing original draft, writing review and editing

RP – Conceptualization, formal analysis, investigation, methodology, supervision

IG – Conceptualization, data curation, formal analysis, investigation, supervision

IP – Conceptualization, data curation, formal analysis, investigation, supervision, validation

Conflict of interest

None to declare.

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RESEARCH ARTICLE

Eating Behaviour and Food Preferences of Tîrgu Mures High School Students

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Introduction. Due to a busy and exhausting urban lifestyle parents do not always have the necessary time to pay sufficient attention to the quality of the dietary habits of their children. **Objective**. Starting from the premise that teenagers have insufficient information about healthy eating, the present study aimed to highlight eating behaviours and nutrition knowledge deficits in a group of 427 high school students from Tîrgu Mures. **Methods**. An observational study based on lifestyle and food frequency consumption was conducted. In 2017, students in fifteen classes from several High School Institutions from Targu Mures, Romania, were asked to complete a questionnaire with questions relating to the current state of health, lifestyle characteristics, anthropometric indicators, frequency of daily meal consumption, significance and intake of food additives, leisure activities performed and also teenagers' preferences for food products. **Results**. The average age of the respondents was 16.1 years old, 72.6% were boys, and 82% lived in the city. 43.6% of respondents stated that food is a necessity, while 22% asserted that food characterizes a pleasure for them. Concerning the calorific value of foods, 32.8% stated that they have no interest in the calorie content of different food products while only 26%, mainly girls, took notice of these. 31.10% of respondents indicated that they include the recommended amount of vegetables in their daily diet, 22% prefer to eat preserved foods while increased consumption of sweets was observed in 39.80%. 55.50% of respondents ate breakfast on a regular basis, and 37% read food labels. **Conclusions**. The results emphasise the necessity to develop more effective educational programs designed to create necessary background information for a young generation, change adolescent dietary behaviours for the better, and thus prevent dietary related diseases.

Keywords: teenagers, nutrition, behavior, food habits

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Introduction

Various factors, such as physical activity, obesity, or stress, are related to school performance and health status [1].

Academic achievement and children's eating habits can be influenced by parental factors such as levels of parenting, children's school performance and family income. Also, nutrition behaviour is one of the most relevant characteristics that can be taken into account when analysing a child's performance levels [1,2].

Neuroendocrine disorders with serious health consequences can results if a child's nutritional needs and daily activities are not properly monitored [3,4].

Nutrients and the derived energy are essential in maintaining the structural and functional integrity of an organism, and their deficiency is reflected in the growth and development of children and adolescents. Children and adolescents require special nutritional needs especially in the full height-weight development stages, which occur from birth to three years of age, pre-puberty and puberty. For example, proteins are the primary plastic factor for cells, carbohydrates also have a plastic role, but they are the primary source of energy for nerve cells and red blood cells. Lipids represent a higher source of energy, with phospholipids being involved in the structure of the cell's membrane and are essential to a cell's function [3,5].

Several studies have highlighted that skipping breakfast has become a habit among adolescents and that it may result in short-term memory [6,7]. This is corroborated by other studies which highlighted the importance and effects of breakfast on school performance in which it has been shown that eating breakfast may be beneficial for normal brain functioning due to the gradual release of energy and the intake of micronutrients, especially iron, iodine and vitamin A.

The daily habit of eating breakfast daily is decreasing among young girls and boys, and Romania students have been shown to eat breakfast on a regular basis much less frequently than students from the US and UK [6,7].

The present study aimed to analyse the risk profile of Tîrgu Mureș teenagers regarding eating behaviours based on dietary habits, frequency of daily meal consumption, significance and intake of food additives, leisure activities undertaken by teenagers, and opinions regarding fast food products versus healthy foods.

Methods

This observational study was conducted between 06.02.2017- and 29.05.2017, in fifteen classes from several High School Institutions from Tîrgu Mures, Roma-

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nia. The sample consisted of 427 students of which 72.6% were boys, aged 15 to 20, with an average of 16+/-2,5, and 27.4% girls, aged 16 to 21, with an average of 16+/-2,5. In the case of some students, the High school normal age of 14-18 years is exceeded by 1-3 years because they have interrupted their studies.

Data collection was carried out by applying a validated multiple-choice questionnaire containing 56 questions, in which respondents could choose from two to six possible answers. Each respondent gave written consent to complete the questionnaire.

By completing the questionnaire, participants had to give information regarding the importance of food in their lives, current state of health, lifestyle characteristics, anthropometric indicators, the frequency of daily meal consumption, the significance and intake of food additives, the leisure activities performed and also their preferences for food products.

Anthropometric measurements were collected and were performed by trained dietitian students using the Tanita Scale (Model: BC 1000) and Seca Portable Stadiometer, and Body Mass Index was calculated by weight(kg)/ height(m)^2.

The statistical analysis was carried out using GraphPad Prism V6. Chi-square test was used to determine if there is a difference between categorical variables. Continuous variables are presented as mean±SD, while categorical variables as absolute frequencies.

The level of significance was set at $\alpha = 0.05$.

Results

Body mass index (BMI) measurements showed that 11% (no=47) of the respondents were underweight, 6.2% (no=27) overweight and 2.5% (no=11) were obese of 1st grade. (Figure 1)

The results showed that 32.8% of those surveyed were not at all interested in the number of calories they consume, 41.2% were not familiar with the number of calories foods contain, and only 26% are concerned about this issue. (Figure 2)

Gender differences in adolescents were statistically significant (X^2=3.56, p<0.05), 72.6% were boys. (Table II)

A significant proportion (63.90%) refuse to eat extra fatty foods, 27.6% said they were accustomed to avoiding tempting meals, and 40.3% do not have a problem with their consumption. Most of the teenagers prefer sweet drinks, coffee, energy drinks even alcoholic ones (63.4%), and 38.7% were drinking sugary drinks day-to-day.

8.7% consumed fast-food products once a month, while 7% of them eat such products 2-3 times a month (Figure 3), statistically insignificant by gender (X^2=5.67, p>0.05) (see Table I).

More than half of pupils (63%) stated that they do not read food labels before buying a product. Concerning buying food, students' families (35.1%) buy them food products according to their preference, while 29.6% were buying based on a shopping list. (Figure 4)

Concerning the consumption of sugar-sweetened beverages and energy drinks, 55.30% respectively 47.70% of the high school students stated that they consume the products mentioned above (Figure 5).

A share of 52% of high school students stated they do not live a sedentary life, most of them used to do physical education and sports at school with a frequency of just 3-4 hours per week. (Table II)

Half of the high school students believe that they need more information about healthy eating, and 44.7% saying they are not being offered enough nutrition information/ advice at school.

Table I. The anthropometric characteristics of studied sample

Parameters	Girls	Boys
Total, n(%)	117(27.4%)	310 (72.6%)
Age (years), mean ± SD	16.78±1.05	16.54±1.09
Weight (kg), mean ± SD	65.21±13.11	66.2±13.62
Height (cm), mean ± SD	171.3±11.4	172.2±11.9
BMI, kg/m^2, mean ± SD	22.9±2.5	23.1±2.8

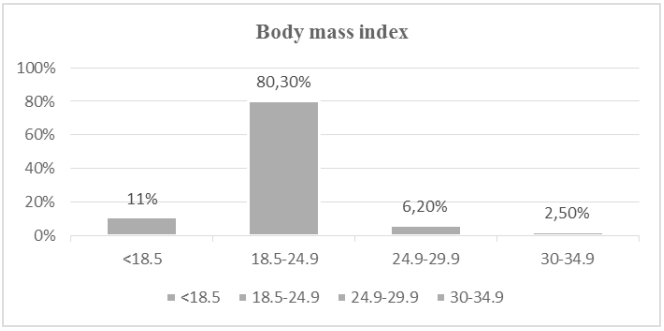

Fig. 1. Body mass index of the studied group

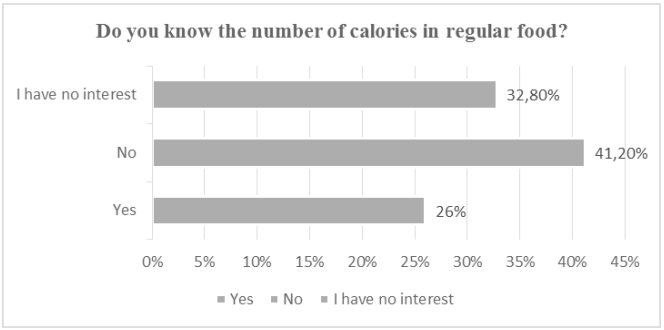

Fig. 2. Knowledge about the number of calories in different foods

Discussion

According to the study "Health Behaviour in School-aged Children", 14% of 11-year-old girls and 21% of 11-year-old boys are overweight or obese. The same situation has been observed in 13-year-old teenagers, but in a slightly different proportion, with 15.8% of girls and 20% of boys suffer from being overweight or obese. In 11-year-olds, 20% of girls and 18% of boys "perceive" themselves to be too fat, whereas in 15-year-olds, 27% of girls and 18% of boys "perceive" themselves to be too fat. In 11-year-olds,

Table II. The relationship between students gender and determinants of food preferences

Question		Girls	Boys	Total	Chi square, p - value
Do you know the number of calories in regular food?	Yes	15.0%	11.0%	26.0%	X2=3.56, p<0.05
	No	20.0%	21.2%	41.2%	
	I have no interest	17.0%	15.8%	32.8%	
How often do you eat at fast food restaurants?	I do not consume	3.0%	16.1%	19.2%	X2=5.67 p>0.05
	Occasionaly	19.8%	25.0%	44.9%	
	Once/month	17.7%	9.13%	26.9%	
	2-3 times/month	4.18%	4.72%	8.9%	
By which criteria your family is buying food?	By price	12.6%	11.7%	23.8%	X2=9.92 p<0.05
	By label	3.98%	7.49%	11.5%	
	Based on a list	5.85%	23.1%	29.6%	
	By preferences	4.91%	30.2%	35.1%	
Do you think that school is providing enough information about nutrition?	Yes	8.43%	28.8%	37.2%	X2=2.3 p>0.05
	No	13.34%	31.38%	44.7%	
	I do not know	5.6%	12.4%	18.0%	
Do you drink sweet beverages?	Yes			55.3%	X2=2.1 p>0.05
	No			44.7%	
Do you consume energy drinks?	Yes	12.17%	35.59%	47.7%	X2=1 p>0.05
	No	15.22%	37.0%	52.3%	

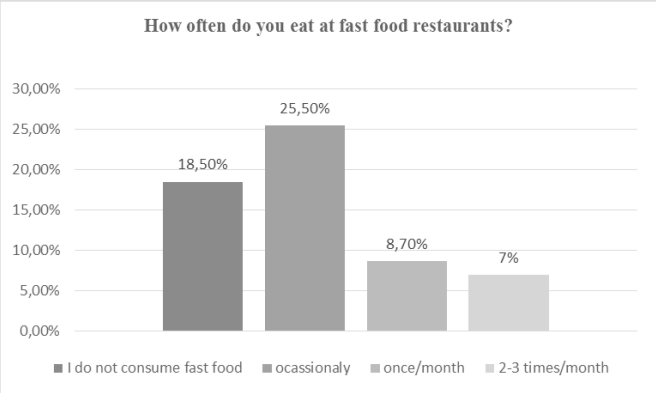

Fig. 3. The frequency of eating fast foods

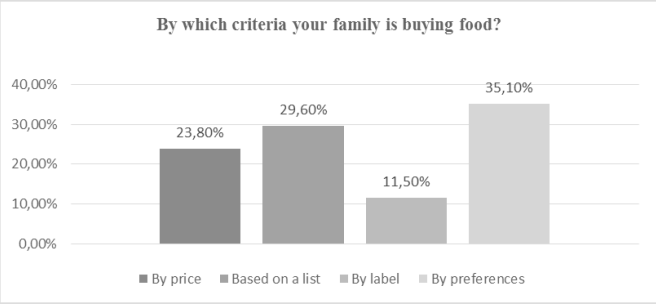

Fig. 4. The motivation to buy food products in the family distribution

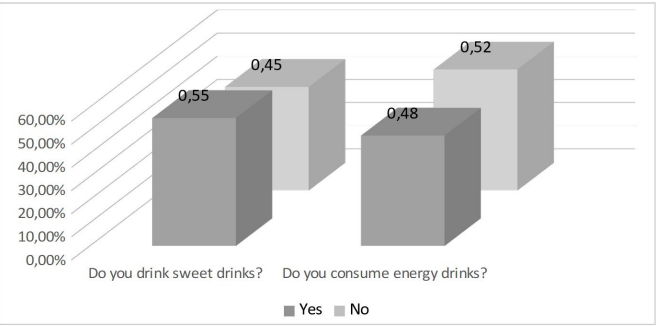

Fig. 5. The consumption of sweet and energy drinks among high school students

14% of girls and 13% of the boys surveyed were undertaking a weight-loss program, and in the 15-year-old age group, 16% of girls and 10% of boys adopted this behaviour [8,9].

The results of the present study confirmed that 10% of the surveyed teenagers are overweight and 11% are underweight. Self-perception of one's body weight is considered to be an important step towards leading a healthy lifestyle. Body image represents a person's perception of his/her own body, and it is importance increases when children reach puberty and face body development changes. Satisfaction about their bodily appearance decreases with age and children often believe they are overweight [8-10].

The survey intended to shed light on high school students' knowledge regarding the number of calories in common foods, as well as on the type of food they prefer to consume: *cooked, raw, processed, semi-prepared.* As a healthy lifestyle is not just keeping track of adolescents' daily calorie intake, another important aspect that was intended to find answers about, when applying this questionnaire, was the frequency of drinking soft drinks, alcohol, coffee as well as teenagers' smoking habits.

Girls are more concerned with their body image, and increased obesity and overweight in industrialized countries have led to increased dissatisfaction with their bodies. There is a significant downward trend of breakfast eating among pupils from the ages of 11 to 13 years with only 55.5% of students taking breakfast, 63.2% consuming lunch, and 56.7% having dinner on a regular basis [10-12]. A similar trend was reported in the current study.

In agreement with similar recent studies, teenagers are not interested in the information on food labels, and they do not count foods calories. Contrarily, they are receptive to lectures, competitions, applications regarding proper diet and weight maintaining methods [11-14].

In recent years, new health education and prevention programs have been implemented on a national level by the Romanian Ministry of Health and Ministry of Education

in order to reduce the risk of excessive fat and sweet food products together with sweet beverages consumption and also to increase the frequency of physical activity among high school teenagers [15-17], with excellent results.

Conclusion

The increased consumption of fast foods and sugar-sweetened beverages confirmed that half of the high school students have an inadequate diet.

Daily breakfast consumption decreases significantly with age, among both girls and boys.

More than half of the students taking part in the survey do not read food labels before buying a product.

About half of Tîrgu Mures high school students taking part in the survey believed they do not live a sedentary life and most of them do physical education and sports at school with a frequency of just two hours per day during the week.

The results emphasise the necessity to develop more effective educational programs designed to change adolescent dietary behaviours in order to prevent related diseases and create necessary background information for a young generation. More community-based food and lifestyle programs need to be implemented for promoting physical activity and healthy lifestyles among teenagers as well as to offer them the necessary education regarding appropriate caloric intake and health risk behaviours.

Authors' contribution

MS – Conceptualization, study design
VR – Drafting, revision
FR – Drafting, final approval
CMD – Data acquisition, revision
IS – Data acquisition, data analysis
LN – Conceptualization, data analysis, final approval

Conflict of interest

The authors have no conflict of interest to report.

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RESEARCH ARTICLE

Effect of Food on the Pharmacokinetics of Gliclazide 60 mg Modified Release Tablet in Healthy Caucasian Volunteers

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Objective: To evaluate the food effect on glicazide disposition in clinical trials conducted on healthy Caucasian volunteers who were given a new modified release oral formulation of Gliclazide 60 mg developed by Sun Pharmaceutical Industries, India. **Methods**: The studies were designed as open-label, randomized, single-dose, crossover studies that consisted of two periods. During each study, venous blood samples were taken before and after drug administration up to 96 hours. Subsequently, individual plasma profiles were determined and non-compartmental method was employed for the assessment of food effect on the pharmacokinetic profile of gliclazide. The statistical significance of differences for the main pharmacokinetic parameters was evaluated by ANOVA test, for $p < 0.05$ statistical significance was decided. The relative profiles of absorption of gliclazide were obtained by mathematical deconvolution. All calculation were performed by Phoenix WinNonlin®. **Results**: High-fat, high-calorie meal decreased gliclazide exposure. The mean maximum plasma concentration decreased with 14%, while the mean total area under the plasma concentration-time profile registered a 17% decrease. The elimination half-lives under fasted and fed conditions were comparable and the time to maximum plasma concentration was shortened under fed condition. Safety evaluation showed that overall gliclazide was well tolerated under both fasted and fed condition. **Conclusions**: The statistical analysis revealed the lack of food effect on the new modified release tablets of Gliclazide 60 mg. However, before stating a definite conclusion regarding the food effect on gliclazide pharmacokinetic profile, additional studies on patients with type 2 diabetes mellitus should be conducted.

Keywords: gliclazide, food effect, pharmacokinetics, clinical trial, healthy Caucasian subjects

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Introduction

Diabetes mellitus (DM) is a major health concern worldwide, which has reached epidemic proportions by currently affecting more than 422 million people around the world, with a prevalence among adults that has risen to 8.5% in 2014 [1,2]. Only in 2015, DM directly caused an estimated 1.6 million deaths, and World Health Organization (WHO) presumes that by 2030 DM will become the seventh leading cause of death worldwide, while the International Diabetes Federation (IDF) forecasts that by 2035 the total number of people suffering of DM will rise to 592 million [1,3]. Type 2 diabetes mellitus (T2DM) is non-insulin-dependent DM accounting for approximately 90 to 95% of all diagnosed cases of DM, and is characterized by insulin resistance in tissues and/or in pancreatic β-cell dysfunction (defects in insulin secretion) [4,5]. Eventually, this leads to progressive hyperglycemia and reduced sensitivity to insulin in tissues such as liver and muscle [6]. The insulin resistance developed in the liver contributes to elevated hepatic gluconeogenesis, while in muscle the glucose uptake is limited [7]. Furthermore, the adipose tissue may exacerbate the development of insulin resistance by secreting inflammatory cytokines (i.e., IL-6 and TNF-α) into the bloodstream and thus lead to the progression of T2DM [8,9]. Long-term hyperglycemia can lead to complications at microvascular level (retinopathy, neuropathy and nephropathy) and macrovascular level (cerebrovascular, cardiovascular and peripheral vascular disease), thus increasing the mortality, morbidity, and healthcare costs and, on the other hand, reducing the quality of life and life span [6,8]. The risk factors for T2DM include obesity, physical inactivity, impaired glucose metabolism, family history of diabetes, tobacco use, race/ethnicity, and recent data suggest the genetic predisposition (more precisely genes CAPN10, PPARG, TCF7L2, KCNJI1, FTO, HHEXIDE, KCNQ1 and MC4R) as a key to susceptibility often correlated with rapid environment changes (for instance, lifestyle factors) [4].

The main objective of T2DM therapy is to reduce hyperglycemia, reach target blood glucose level and maintain a glycosylated hemoglobin (HbA1c) concentration of ≤ 7%, as recommended by The American Diabetes Association, or even ≤ 6.5 % if it can be achieved in an affordable and safe manner [6,10]. These objectives are attained by elevating plasma insulin levels (by administering oral agents that promote insulin secretion or by direct insulin administra-

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tion), improving insulin sensitivity of tissues, and reducing the extent of carbohydrate absorption from the gastrointestinal tract [5]. These therapeutic effects can be achieved through insulin secretaogues (for instance, sulphonylureas such as gliclazide, or meglitinides), insulin sensitisers (e.g. thiazolidinediones), and external insulin delivery (insulin analogues) [4,6].

Gliclazide is an oral hypoglycemic agent indicated for the treatment of T2DM. It binds to β-cell sulfonylurea receptor and blocks K_{ATP} channels, leading to depolarization of β-cells and decreased potassium efflux, thus opening the voltage-dependent Ca^{2+} channels resulting in calmodulin activation, and eventually leading to release of insulin-containing secretory granules [11]. As gliclazide belongs to the second generation class of sulphonylureas, it is widely used as the second-line recommended treatment of hyperglycemia after metformin, or in combination with it when metformin alone does not suffice to control blood glucose level [6,12,13]. It displays affinity only for sulphonylurea receptors localized in β-cell type (SUR1), smooth muscle and adipose tissue (SUR2B) [6]. Studies have reported that sulphonylureas can reduce the Hb1Ac levels by around 1.51% if used as monotherapy, or by approximately 1.62% when used in combination with oral diabetes treatment [6]. Considering the advantages of the second-generation sulphonylureas, it is very likely that gliclazide is commonly prescribed for the treatment of T2DM worldwide.

Gliclazide displays rapid and complete absorption after oral administration, with inter-individual variability regarding the time to reach the peak plasma concentration (t_{max}). Also, age related differences were observed for this parameter and for peak plasma concentration (C_{max}), which is attained within 4-6 h after oral administration [11]. Steady-state concentration of gliclazide is attained after 2 days administration of 40 to 120 mg, with increased C_{max} and t_{max} after multiple doses. A low volume of distribution (13 to 24 L) due to high plasma protein binding affinity (85 to 97%) characterizes gliclazide [11]. It is extensively metabolized to 7 metabolites mainly in the liver, primarily by CYP2C9 and to a lesser extent by CYP2C19 and CYP2C18 [14]. The kidneys (60-70%) and feces (10-20%) eliminate its metabolites and conjugates. The hypoglycemic effect of gliclazide can display individual differences because of CYP2C9 polymorphism in addition to pharmacodynamics factors [14,15]. The half-life of elimination ($t_{½}$) varies from 8.1 h to 20.5 h after administration of 40 to 120 mg p.o. The initial dosage of gliclazide is 40 mg per day and can be increased to 320 mg daily, depending on the severity of glycaemia and disease state [11]. Modified release pharmaceutical formulations which exist already on the market release gliclazide through a gel layer which is formed as a consequence of the hydration of the tablet, thus assuring a prolonged release of the active substance for a better glycaemia control, lesser tablet administrations per day, high compliance and adherence to treatment of T2DM patients [16,17].

In Romania, approximately 12.4% of the population was diagnosed with diabetes, according to the 8th Edition of International Diabetes Federation (2017), many of them currently being under medication with hypoglycemic agents such as gliclazide [18]. A new modified release oral tablet of *Gliclazide 60 mg* was developed *by* Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India. The formulation was proved bioequivalent with Diamicron® MR (Servier, France) in a previous study conducted at the Clinical Pharmacology and Pharmacokinetics Department of Terapia SA, Romania [19]. The aim of the study presented in this article was to evaluate the food effect on gliclazide pharmacokinetics in healthy Caucasian volunteers by comparing the data obtained in two bioequivalence study of the newly developed tablet with Diamicron® MR (Servier, France), conducted under fasted and fed condition of subjects.

Materials and methods

Subjects

The clinical trials were conducted in accordance with US 21 CFR Part 320, the ICH E6 (R1), Good Clinical Practice guidelines and the principles of Helsinki (1964) and its amendments (Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000, Washington DC 2002, Tokyo 2004, Seoul 2008 and Brazil 2013). The study protocols were approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Hatieganu", from Cluj-Napoca (Romania) and by the National Agency for Medicines and Medical Devices, Romania. A written informed consent was obtained from each volunteer prior to performing any procedures related to the clinical studies.

The studies took place at the Clinical Pharmacology and Pharmacokinetics Department of Terapia SA, Romania.

Forty-eight Caucasian males and females were enrolled in the fasted study, the Reference data of the assessment, while twenty-six healthy volunteers were enrolled in the fed study, the Test data of the assessment. Inclusion criteria required the study population to be healthy, nonsmoking Caucasian females or males, aged between 18 and 55 years. Their health status was evaluated according to their medical history, physical examination, electrocardiogram (ECG) and routine laboratory investigations (biochemistry, hematology, urine and serological tests). In case of female volunteers, a pregnancy test was carried out at screening, prior to admission in each study period, as well as at the end of the clinical trial. The volunteers were also tested for hepatitis B surface antigen, anti-HIV, anti-hepatitis C antibody, and in case of positive results they were further excluded from the trial. Also, subjects were excluded from the study if they were smokers, they had a history of drug or alcohol abuse, a history of documented allergy, a hypersensitivity to gliclazide, or if they were under regular medication. Exclusion criteria were also considered any medical

condition, dietary products or lifestyle factors that could influence drug response. Drug intake was evaluated for 28 days prior to studies in order to avoid any drug-drug interaction or enzymatic induction/inhibition.

The number of subjects for each study period was assessed based on: test/reference ratio in the range of 95%-105% for fasting conditions and respectively 90%-110% for fed conditions, an approximately 30% expected coefficient of variation in fasting condition and respectively 15% in fed conditions, power of 80%, level of significance of 0.05, bioequivalence interval 0.8 -1.25 using SAS software version 9.1.3 and the possible withdrawals and/or dropouts were also taken into consideration.

Study design and drug administration

The data were collected from two bioequivalence studies; each consisted in gliclazide administration with or without food [19]. Each study was designed as an open-label, randomized, single-dose, crossover study that consisted of two periods during which the subjects were given the test product developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India, and reference product Diamicron® MR, Servier, France [19]. For the current assessment of the food effect on the pharmacokinetics of gliclazide, the Reference data was considered the study conducted under fasted condition, while Test data was elected the study conducted under fed state of subjects; in each study the new formulation Gliclazide 60 mg developed by Sun Pharmaceutical Industries, India, was administered to every subject.

During the fasted study, Reference data, the test product was administered to all volunteers after a fasting period of at least 10 h. The drug (gliclazide 60 mg) was taken as a single-dose under medical supervision and trained study personnel with 240 mL of 20% glucose solution. After drug administration, 60 mL of 20% glucose solution were given to each volunteers every 15 min for up to 4 h post-dose in order to avoid hypoglycemia. Moreover, post-dose no food intake was allowed for at least 4 hr. Furthermore, subjects were allowed to drink water only with at least 1 h pre-dose and starting 2 h post-dosing. During their 46 h confinement at the Clinical Pharmacology and Pharmacokinetics Department of Terapia SA, Romania, they were provided with identical standardized meals at the same hours in each study period. All healthy volunteers were given gliclazide while seated and were carefully instructed to remain seated or in semi-supine position for 2 h post-dose.

The study protocol and drug administration for the fed study (Test data) was similar to the Reference period, with the following difference: after a 10 h fasting period, all subjects were given a high-fat, high-calorie standard meal, 30 minutes before drug administration and the confinement was of 46.5 h. Thus, during the Test period, all volunteers were under fed condition, while during the Reference period, the pharmacokinetic profile of gliclazide 60 mg was obtained under fasting condition.

The pharmaceutical product used was Gliclazide 60 mg, a new modified release oral tablet developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India.

Blood plasma samples collection and bioanalytical methods

During the Reference period, venous blood samples (4 ml) were drawn prior to drug administration and at 1, 2, 3, 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 15, 16, 20, 24, 36, 48, 72 and 96 hours after drug administration. For the Test period, the sampling design was the following: pre-dosing and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 20, 24, 36, 48, 72 and 96 hours post-dose. The blood samples were collected in K_3-EDTA vacutainers, centrifuged for 15 min at 4000 rpm under refrigeration and the separated plasma was recovered and kept at -50°C until analysis.

The plasma samples were prepared for analysis as it follows: over 150 µL of plasma were added approximately 50 µL of internal standard dilution (Gliclazide D4, concentration 1500 ng/mL) and 150 µL of water and the mixture was vortex mixed. Afterwards, the solid-phase extraction of the samples was carried out in cartridges (type Cleanert PEP-H 30 mg/mL), which were previously conditioned with 1 mL of water and 1 mL of methanol. Consequently, gliclazide was extracted by evaporation under nitrogen steam by using methanol and the obtained residue was reconstituted with 200 µL of mobile phase. All samples were processed in the previously presented manner under low light conditions.

Table I. Performance of the 2 tests

Center	Moment	Specificity	CI95%	PPV	CI95%	P
Târgu Mureș	Clonidine 30 minutes	6.82%	1.43-18.46%	57.29%	46.78-67.34%	<0.08
	Clonidine 60 minutes	86.36%	72.66-94.82%	90.16%	79.81-96.31%	<0.0001
	Clonidine 90 minutes	61.36%	45.44-75.65%	76.39%	64.9-85.63%	<0.0001
	Clonidine 120 minutes	25%	13.19-40.29%	62.5%	51.56-72.56%	<0.0001
	Insulin 30 minutes	75%	19.42-99.37%	95.83%	78.86-99.89%	0.0014
	Insulin 60 minutes	50%	6.76-93.24%	92%	73.96-99.02%	0.0171
	Insulin 90 minutes	50%	6.76-93.24%	92%	73.96-99.02%	0.0171
	Insulin 120 minutes	25%	0.63-80.58%	88.46%	69.86-97.55%	0.1481
Bucharest	Clonidine 60 minutes	89.1%	77.7-95.9	95.16	89.7-98.2	<0.0001
	Clonidine 120 minutes	18.2%	9.1-30.9	72.4	64.9-79.1	<0.0001
	Insulin 30 minutes	68.4%	43.4-87.4	92.3	84.01-97.1	<0.0001
	Insulin 60 minutes	42.1	20.2-66.1	85.7	75.9-92.6	<0.0001

A validated high-throughput liquid chromatography tandem mass spectrometry method (LC-MS/MS) using Gliclazide D4 as internal standard was employed to determine the plasma concentrations of gliclazide. The chromatographic system was an Agilent 1200 series (binary pump, autosampler, thermostat, from Agilent Technologies®, Santa Clara, CA, USA) coupled with a triple quadrupole mass spectrometer API 3200 (from Applied Biosystem MDS SCIEX®, Framingham, MA, USA). The chromatographic column used was a BDS HYPERSIL C18 (50 mm × 4.6 mm, 3 µm, from Thermo Fisher Scientific Inc., USA) and the mobile phase consisted of a mixture of water/methanol/formic acid 98%– 100% [100:900:0.01 v/v/v]. The flow rate was 0.8 mL/min, the thermostat temperature was set at 35°C, the injection volume was 10 µL, and the run time was 2.5 min. The mass spectrometry detection was in multiple reaction monitoring mode, positive ions, using Turbo Ion Spray as ionization source. The monitored ions transitions were m/z of 324.10→127.10 for gliclazide and 328.10→127.10 for Gliclazide D4 as internal standard. The retention times for gliclazide and internal standard were between 0.3-2 min. Gliclazide concentrations for each sample were determined from peak area ratios of gliclazide and internal standard and the calculations were performed by the Analyst software version 1.4.2. The quantification limit was 5.00 ng/mL. The analytical method was validated in terms of specificity, linearity, between- and within-run precision and accuracy, and analyte recovery. The calibration curve of gliclazide and internal standard were linear between 5-5016.48 ng/mL. The between-run accuracy was in the interval 90.53%-110.14%, the within-run accuracy was from 89.10% to 114.98%, the between-run precision was 1.83%-4.69%, and the within-run precision was 0.96%-4.38% [19].

Pharmacokinetic analysis

The pharmacokinetic parameters of gliclazide, when given under fast or fed condition of subjects, were estimated by a non-compartmental analysis method, performed by using Phoenix WinNonlin® PK version 6.3 (Pharsight Co., Mountain View, Calif., USA). The following pharmacokinetic (PK) parameters were determined: the maximum plasma concentration (C_{max}, ng/mL) and the time to reach C_{max} (t_{max}, h) were obtained directly from evaluating the values obtained experimentally, according to the non-compartmental analysis. The area under the plasma concentration–time curve from time zero to the last quantifiable concentration (AUC_{0-t}, ng*h/mL) was calculated by using the linear trapezoidal rule. The total area under the curve ($AUC_{0-\infty}$, ng*h/mL) was obtained by adding to AUC_{0-t} the report value of the last measurable concentration divided by the elimination rate constant (C_t/k_{el}). The constant of elimination (k_{el}, h^{-1}) was estimated by log-linear regression analysis of terminal portion of the plasma concentration-time profile. The half-life time of gliclazide ($t_{½}$, h) was calculated using the formula $t_{½} = 0.693/k_{el}$. Other determined pharmacokinetic parameters were the apparent clearance (Cl_F, mL/h) and the apparent volume of distribution (Vd_F, mL), estimated by taking into account the values of k_{el}. Moreover, with the purpose of obtaining the absorption profile of gliclazide from the site of administration (gastrointestinal tract is the main site of absorption after oral administration of the novel modified release tablet), the data obtained from plasma samples were further analyzed by mathematical deconvolution, thus obtaining the relative fraction of absorbed gliclazide over time.

Statistical analysis

The statistical analysis was performed by using Phoenix WinNonlin® PK version 6.3 (Pharsight Co., Mountain View, Calif., USA). For the comparison of gliclazide's plasma profiles under fasted and fed conditions, the analysis used Type III sum of squares from analysis of variance (ANOVA). A p value less than 0.05 determined for the differences of the PK parameters in-between study periods was considered statistically significant, whilst for t_{max} the non-parametric Friedman test was elected for assessment of differences.

Safety evaluation

Safety evaluation was conducted throughout both clinical trials and consisted of monitoring any adverse event or change in the volunteers' health condition which could be attributed to the given medication, gliclazide respectively. For safety reasons, the glycaemia levels were measured before drug administration and post-dose at 1, 2, 3, 6 and 7 h in both studies. The vital signs (axillary body temperature, sitting blood pressure, and radial pulse) were measured predosing and after drug intake at 4, 8, 12, 24, 36, 48, 72, and 96 h during both studies. For at least 24 h following gliclazide administration in each study period, either the principal investigator or a subinvestigator was available at the Clinical Pharmacology and Pharmacokinetics Department of Terapia SA, Romania, the site of investigation. Moreover, basic clinical examinations including hematological tests, biochemistry and urine analysis were carried out at the end of the study. Likewise, for female subjects the pregnancy test was repeated after the end of the clinical trial.

Results

Subjects

The demographic data of the healthy volunteers who were selected for the clinical trial are shown in Table I. For the Reference period, a number of 48 subjects were enrolled of which 41 completed the study. For the Test period, 26 subjects were selected of which a number of 23 finalized the trial without any protocol deviations.

Pharmacokinetic analysis

Mean plasma concentration-time profiles of gliclazide, when given under fasted or fed state, are presented in Figure 1.

Table I. Demographic characteristics of the subjects included in the study

Characteristic	Reference period (fasted state)	Test period (fed state)
Number of subjects	41	23
Gender (number) – Men	31	23
-Women	10	0
Age (years) – mean (SD)	23.1 (4.5)	24.7 (5)
Range	19-40	19-35
BMI** (kg/m2) – mean (SD)	23.5 (3.1)	22.9 (2.9)
Range	18.67-28.90	18.6-28.7

*SD – standard deviation; **BMI – body mass index

Following the graphical representation of plasma concentration profiles for gliclazide, the mean pharmacokinetic parameters, when given in fasted state of subjects or when the subjects were under fed state are shown in Table II.

The food effect findings reported in this study indicate that gliclazide exposure was lower after food intake. The values of C_{max} were by ~14% lower under fed condition (2066.11 ± 925.43 ng/mL vs 1800.17 ± 405.20 ng/mL) and $AUC_{0-\infty}$ registered a ~17% decrease during the fed state study period (38849.79 ± 20018.59 ng*h/mL vs 33041.37 ± 11075.75 ng*h/mL). The time to reach the peak plasma concentration (t_{max}) was shorten from 9.15 ± 2.41 h under fasted condition to 8.95 ± 2.10 h under fed state of the subjects. Likewise, the time from the administration to the beginning of absorption (t_{lag}) recorded a 3.3% decrease after food intake, from 2.51 ± 1.40 h to 2.43 ± 1.16 h. On the other hand, the half-life time of gliclazide ($t_{½}$) increased with ~5% during Test period, from 14.42 ± 4.63 h (fasted state) to 15.27 ± 4.60 h (fed state), even though the constant of elimination did not display any modification during the study (k_{el}=0.05 h^{-1}).

After the mathematical deconvolution of the plasma concentrations of gliclazide, the relative fraction absorbed from the site of administration over time was obtained and is depicted in Figure 2.

The relative profile of gliclazide's systemic absorption confirmed the t_{lag} of approximately 2.5 h after drug administration. Afterwards, plasma levels increase progressively, from 3 to 24 hours post-dose 80% of gliclazide is systemically absorbed. Its absorption continues with almost constant rate up to 96 hours post-dosing resulting in a plateau shaped curve, when the entire given dose is completely absorbed in the general circulation from the small intestine.

Statistical analysis

The statistical evaluation by ANOVA test was performed with the purpose of investigating the existence of a statistically significant difference between the mean values of the main pharmacokinetic parameters of gliclazide within study periods and the results are summarized in Table III. For t_{max} differences evaluation, the non-parametric Friedman test concluded the lack of statistical significance of the registered differences under fasted versus fed state of the subjects.

The statistical evaluation by ANOVA test did not conclude any statistically significant difference for the evaluated pharmacokinetic parameters between studies, therefore food intake did not have a major impact on gliclazide's disposition in the body.

Safety evaluation

A summary of the adverse event monitored during both study periods, after administration of 60 mg gliclazide p.o., is given in Table IV.

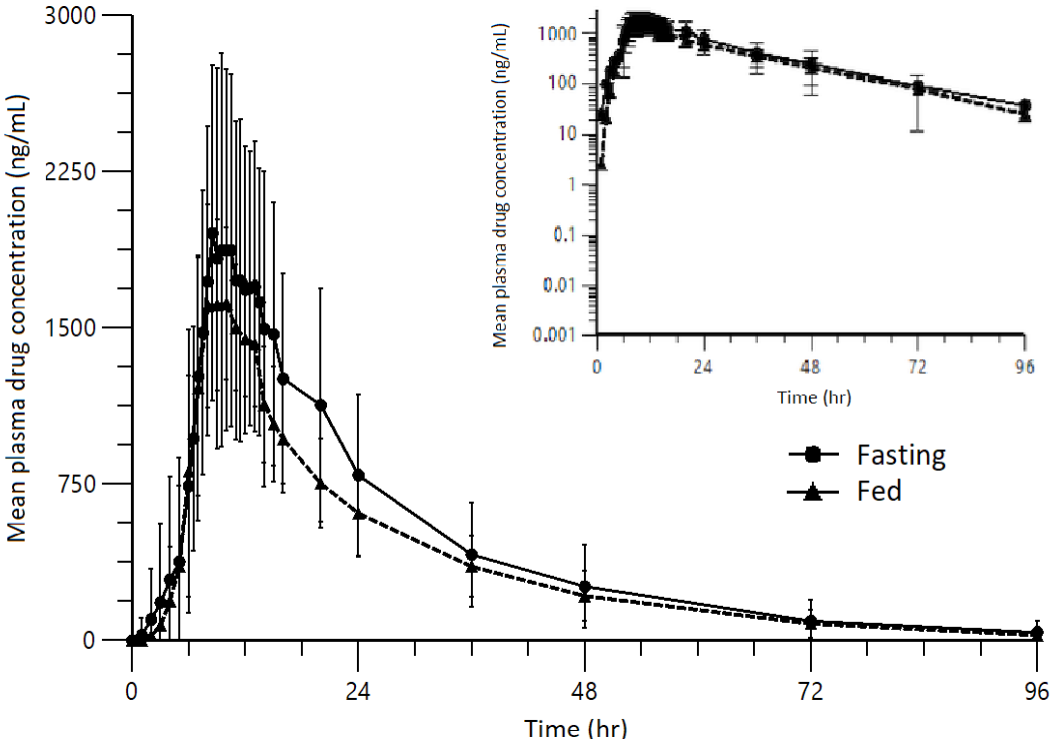

Fig.1. Mean ± standard deviation (SD) plasma concentration-time curves of gliclazide (60 mg, p.o.) administered in fasting state (n=41) or fed state (n=23). Insert: semi-logarithmic presentation

Table II. Summary of pharmacokinetic (PK) parameters of gliclazide after a single-dose of 60 mg p.o. administered under fasted or fed state of the subjects

PK parameter (units)	Study period							
	Reference (fasted state)				Test (fed state)			
	Geometric mean	SD	Median	CV%	Geometric mean	SD	Median	CV%
Cmax (ng/mL)	2066.11	925.43	2093.95	41.59	1800.17	405.2	1885.76	21.97
tmax (ng/mL)	9.15	2.41	8.5	25.52	8.95	2.10	8.00	22.92
AUC0-t (ng*h/mL)	38195.09	20343.49	37148.24	47.94	32479.33	11338.14	32541.45	33.16
AUC0-∞ (ng*h/mL)	38849.79	20018.59	37630.76	46.67	33041.37	11075.75	33162.09	31.96
kel (h-1)	0.05	0.02	0.05	29.91	0.05	0.01	0.05	25.04
t½ (h)	14.42	4.63	14.16	30.69	15.27	4.60	14.54	29.05
tlag (h)	2.51	1.40	3.00	55.67	2.43	1.16	2.00	47.68
MRT (h)	24.73	6.10	23.89	24.01	25.86	6.25	25.77	23.57
Cl_F (mL/h)	1511.12	716.91	1585.08	43.03	1769.72	617.79	1785.92	33.16
Vd_F (mL)	31427.84	7547.70	31502.83	23.39	38994.82	8344.65	37839.01	20.96

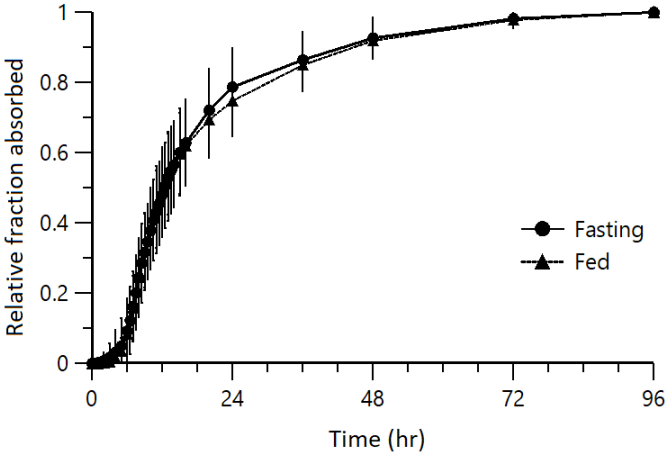

Fig. 2. The relative fraction absorbed of gliclazide (60 mg, p.o.) in the systemic circulation from the site of administration over time in subjects under fasted (n=41) or fed state (n=23)

A total of 15 treatment-emergent adverse events were reported by 18 subjects out of 64 (11 under fasted condition and 6 under fed condition, while 2 side effects were reported in both studies, namely hypoglycemia and increased direct bilirubin). All the reported adverse events were of mild-to-moderate intensity and all subjects recovered without sequelae. The most frequently reported side effect was hypoglycemia, which was expected considering it is widely reported after gliclazide administration in therapy [11]. However, in both trials gliclazide was generally well tolerated by subjects, with no severe adverse events leading to withdrawal from the study.

Table III. Statistical analysis results of the mean pharmacokinetic (PK) parameters comparison between Reference period (fasted state) and Test period (fed state) for gliclazide

PK parameter	Units	F_stat[a]	p_value[b]
C_{max}	ng/mL	2.48	0.1202
AUC_{0-t}	ng*h/mL	2.21	0.1425
$AUC_{0-\infty}$	ng*h/mL	2.36	0.1296
k_{el}	h^{-1}	0.58	0.4484
$t_{½}$	h	0.58	0.4484
t_{lag}	h	0.71	0.4035
MRT	h	0.57	0.4545
Cl_F	mL/h	2.09	0.1530
Vd_F	mL	13.63	0.6550
t_{max}	h	Friedman	NS[c]

[a]F_stat – statistic factor; [b]p < 0.05 statistically significant; [c]NS – statistically non-significant

Discussion

Every time a new modified release formulation is evaluated for market release, apart from the bioequivalence study itself, current guidelines recommend a food intervention study on healthy volunteers to investigate the food-drug interaction, considering that food intake may induce physiological changes in human body related to digestion process [20,21]. The administration of a new drug product with food may alter the drug's absorption and may change the bioavailability of the given active substance by influencing either the drug product or the drug substance (release or dissolution) [20,21]. Consequently, the general recommendations for use after such studies are conducted are listed in the drug product's specification file and may vary from take without food, take with food, or take regardless to food intake (with or without food) [22].

In this clinical trial, the pharmacokinetic parameters of gliclazide (single-dose of 60 mg, new modified release formulation, p.o., developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India) under fasted and fed state were determined, as well as the absorption profile from the gastrointestinal tract.

Considering that assessment of the food effect on the extent and rate of a drug's absorption is part of the development process of a new orally administered drug product, numerical deconvolution of the experimental data was used in order to obtain the relative fraction absorbed by gliclazide at the specified time points [20-22]. Gliclazide is a drug belonging to BCS class II, with high permeabil-

Table IV. Summary of the adverse events reported for both periods of the clinical trial

Reported adverse events after gliclazide 60 mg administration p.o.	
Reference period (fasted state, 41 subjects)	Test period (fed state, 23 subjects)
Hypoglycemia	Hypoglycemia
Diarrheic syndrome	Increased blood urea nitrogen
Nausea	Increased alanine aminotransferase
Flu-like syndrome	Increased direct bilirubin
Headache	Increased total bilirubin
Acute gastroenterocolitis	Leukocyturia
Proteinuria	
Increased lactate dehydrogenase	
Increased direct bilirubin	
Urinary infection	
Increased aspartate aminotransferase	

ity and intermediate solubility that delays the absorption rate and onset of action, but with non-solubility-limited absorption after oral administration [23]. This delay in absorption can be attributed to several factors such as: slow disintegration or even non-disintegration of the modified release tablet in correlation with biphasic gastric emptying or absorption of gliclazide from two distinct sites within the upper gastrointestinal tract [24]. This hypothesis can be supported by the fact that gliclazide is an ampholyte with pH-dependent solubility in the gastrointestinal pH range [23]. The absorption profile of gliclazide displayed almost complete superposition under fasted and fed condition of subjects, thus highlighting the optimal and constant release of gliclazide (60 mg, p.o.) from the new modified release formulation developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India. The outcomes of this research are consisted with those previously reported in the scientific literature [19].

Conclusion

In these clinical trials, food was demonstrated to exhibit no clinically or statistically meaningful effect on the bioavailability of gliclazide, given as a single-dose in healthy volunteers. Food effect was demonstrated to not have a statistically significant influence on the overall (extent and rate) exposure to gliclazide, even though a minor decrease in the peak plasma concentration and total area under the plasma concentration-time curve was observed. The reported adverse events during the study are common in this class of oral hypoglycemic agents and were anticipated, however gliclazide proved a favorable safety profile in healthy subjects under fasted or fed condition. These results suggest that gliclazide 60 mg new modified release tablet developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India, could be safely administered to non-insulin dependent (type 2) diabetic patients without regard to food intake according to individual tolerance and patient preference. However, before stating a definite conclusion regarding the food effect on gliclazide pharmacokinetic profile, additional studies on T2DM patients should be conducted.

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Authors' contribution

DP – Study protocols, study design, data analysis, manuscript writing
AMG – Data analysis, manuscript writing
MO – Data collection, safety monitoring
AM – Study design, data analysis, review the manuscript
SB – Bioanalytical analysis, review the manuscript
AK – Study design, review the manuscript
RK – Study design, review the manuscript
LV – Data analysis, review the manuscript

Conflicts of interest

Diana Pop, Adriana Marcovici, Monica Oroian, Sandeep Bhardwaj, Arshad Khuroo and Ravi Kochhar were employees of the Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India, during the conduct of this study.

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CASE REPORT

Uncommon Finding of a Gastrointestinal Stromal Tumor in a Patient with Hyperechoic Liver Lesions - Case Report

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Introduction: Hyperechoic liver lesions identified by conventional ultrasonography are diverse in underlying pathology and most of the time require further investigations. Gastrointestinal stromal tumors (GIST) are rare neoplasms of the gastrointestinal tract which are uncommonly found in metastatic stages at first presentation. **Case report**: We present the case of a 51 years old woman with nonspecific symptoms in which conventional ultrasonography showed hyperechoic lesions in the right lobe of the liver with a diameter up to 40 mm. Esophagogastroduodenoscopy revealed a submucosal tumor on the small curvature of the stomach, on the anterior wall, with central ulceration, with normal narrow band imaging (NBI) mucosal pattern and negative gastric biopsy. Contrast enhanced ultrasonography was performed, describing multiple lesions with inhomogeneous enhancement in the arterial phase and rapid washout at the end of arterial phase. Endoscopic ultrasound with fine needle aspiration (EUS-FNA) biopsy examination was definitive for the final diagnosis of epithelioid gastric gastrointestinal stromal tumor. The patient was diagnosed with T2N0M1 epithelioid gastric GIST, stage IV, and is currently under treatment with tyrosine kinase inhibitors. **Conclusions**: GIST represent a diagnostic challenge in medical practice because of its size, unusual location in the submucosal layer and lack of symptoms. The role of EUS-FNA is of paramount importance in increasing the accuracy of diagnosis in the case of GIST. The particularity in our case consists of the unusual presentation with the lack of specific symptoms and signs associated with the presence of metastatic lesions at the moment of the diagnosis of GIST.

Keywords: gastrointestinal stromal tumors, endoscopic ultrasound, fine needle aspiration biopsy

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Introduction

Hyperechoic liver lesions identified by conventional ultrasonography are diverse in underlying pathology, and usually require careful examination and additional investigations in order to improve diagnostic accuracy [1].

Gastrointestinal stromal tumors (GIST) represent rare neoplasms of the gastrointestinal tract [2], with a reported incidence in most studies between 10-15 cases per million, with an equal distribution between male and females, and a peak incidence in the sixth decade [3].

Case report

A 51 year-old woman was referred to our department for abdominal pain in the right upper quadrant, asthenia, moderate weight loss in the last six months, with no other associated alarm symptoms, and a negative family and personal medical history.

Laboratory values showed no pathological changes. Conventional ultrasonography was performed which showed hyperechoic lesions in the right lobe of the liver with a diameter up to 40 mm, most of them with indistinct margins and peripheral halo, alongside rounded hyperechoic lesions with distinct margins (Figure 1).

The patient underwent an esophagogastroduodenoscopy which showed a submucosal tumor on the small curvature of the stomach, on the anterior wall, with central ulceration and no signs of active bleeding (Figure 2). Narrow band imaging (NBI) examination was performed, describing normal mucosal pattern. Biopsy specimen from the lesion showed normal gastric mucosa.

Total colonoscopy was performed in order to rule out a primary lesion which could be linked to the liver lesions, with no pathological findings being described on the examined tract.

In order to increase diagnostic accuracy, contrast enhanced ultrasonography was performed, describing multiple lesions with inhomogeneous enhancement in the arterial phase and rapid washout at the end of arterial phase (Figure 3). In the venous and parenchymal phase the lesions were hypoechoic.

Ultrasound guided conventional liver sample biopsy from the hyperechoic lesions was inconclusive, without any signs of malignancy or positive immunohistochemistry staining from the selected specimen.

The patient was afterwards referred to endoscopic ultrasound with fine needle aspiration (EUS-FNA) biopsy. The tumor, originating in the muscularis propria, with a diameter of 17/17 mm was described on the anterior wall of the gastric corpus. Biopsy specimens from the gastric lesions put the diagnosis of epithelioid gastric gastrointestinal stromal tumor with low mitotic rate (gastric foveolar epithelium and cells with oval nuclei, and eosinophil cyto-

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plasm, with irregular margins and rare mitosis (1-2), without any signs of central necrosis) The hepatic lesions were described as having simillar morphology. Immunostaining examination of both gastric and hepatic lesions showed intense positivity for c-KIT, DOG1, CD34 and negativity for SMA, S100 markers.

Contrast enhanced computed tomography of the chest, abdomen and pelvis was performed and ruled out local lymph nodes involvement or other sites of metastasis.

Finally, the patient was diagnosed with T2N0M1 epithelioid gastric GIST, stage IV, according to GIST pathology staging and prognostic group classification. She is currently under treatment with tyrosine kinase inhibitors (Imatinib mesylate) and presents with a stationary evolution.

Discussions

GISTs represent the most common sarcoma of the gastrointestinal tract neoplasms, associated with the activation of gene mutation such as KIT and PDGFRA [4]. Sporadic GISTs represent 95% of cases and have been associated with an increased incidence of other cancers such as prostate, breast, small intestine or colorectal cancer [5]. GISTs in direct association with other pathologies are described in syndromes such as neurofibromatosis type 1, Carney's triad (gastric GIST, paraganglioma, pulmonary chondroma), or familial GIST syndrome [6].

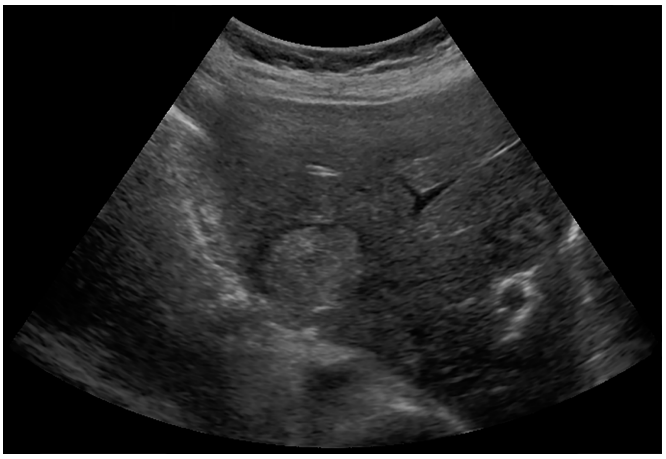

Fig. 1. Conventional ultrasonography view of the liver lesions

Focal liver lesions, especially in non-cirrhotic patients, are detected in most of the cases incidentally or during follow-up for a primary neoplasm, usually at conventional ultrasound [7]. Contrast-enhanced ultrasound (CEUS) has improved the detection rate of diagnosis, with a reported sensitivity and specificity ranging from 80-95% [8]. Malignancies are usually described in CEUS as hypoechoic lesions. Metastases are described as hypo- or hypervascular lesions, the difference being noted at the enhancement during the arterial phase [9].

The particularity in our case is the description at conventional ultrasound of both hyperechoic lesions, with indistinct margins and peripheral halo as well as rounded lesions with more distinct margins. CEUS described hypovascular lesion with weak enhancement in the arterial phase and rapid washout at the end of arterial phase with no lesions with progressive centripetal fill in arterial phase and a persistent hyperechogenicity in late-phase suggesting hemangiomas.

Esophagogastroduodenoscopy has a high detection rate in esophageal and gastric carcinoma [10], but less in GIST due to its submucosal localization, which impairs the possibility of obtaining conventional endoscopic biopsy.

Consequently, endoscopic ultrasound with fine needle aspiration (EUS-FNA) is recommended when gastric GISTs are found incidentally and has emerged as a helpful mean of sampling potential GISTs.

Alongside sonographic features provided by EUS, which supplies significant differential diagnosis data, FNA biopsy is essential for specimen analysis and immunohistochemistry studies [11].

Morphological patterns associated with GISTs are spindle cell, epithelioid or mixed subtype and require immunohistochemical stains in order to confirm the suspected

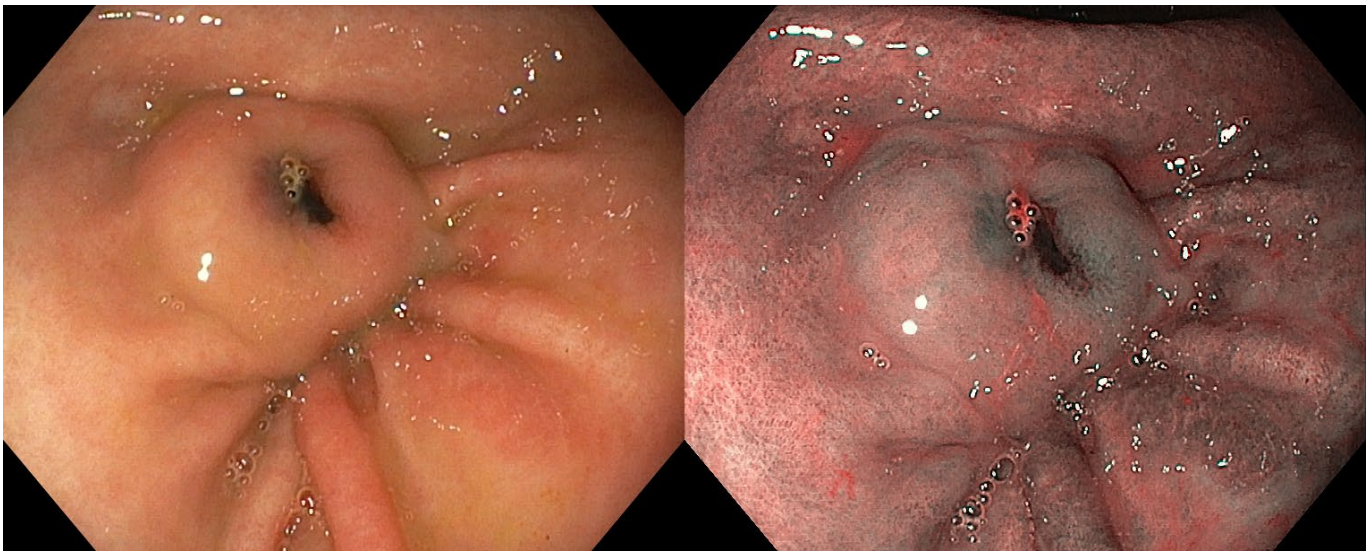

Fig. 2. Endoscopic views of the submucosal tumor - normal view (right) and Narrow Band Imaging (NBI) examination (left)

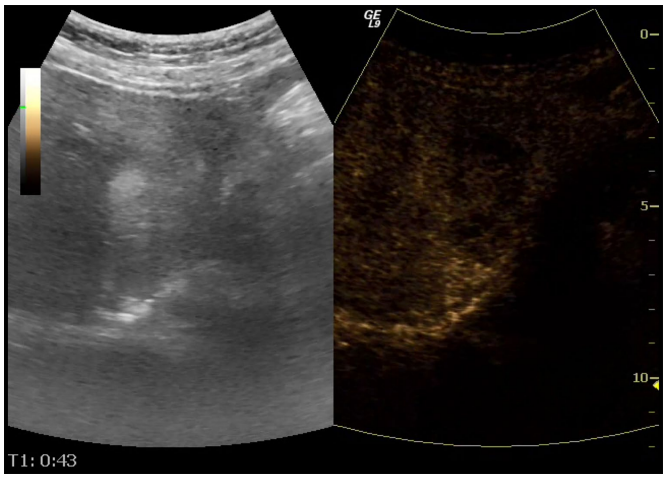

Fig. 3. Contrast enhanced ultrasonography view of the liver lesions - arterial phase

diagnosis. Most of the tumors are positive for c-KIT and anoctamin 1, also known as DOG1, being the most sensitive and specific markers for GIST [12,13].

The first line treatment of GIST in patients, including the cases with resectable metastatic tumors, is surgery or endoscopic resection [2].

Liver and lymph nodes metastases have been reported in published data [14], and represent a major determinant of patient survival and a challenge in the management of GIST treatment.

Abuzakhm et al. describe a case of gastrointestinal stromal tumor located in the jejunum associated with liver metastasis discovered a short period after the diagnosis of GIST [15].

Burkill et al. state in their study the increased incidence of liver or peritoneum metastasis, but usually discovered during follow-up after surgical resection of GIST. Liver metastasis were associated with GIST who presented increased size at diagnosis or the histopathological presence of central necrosis [16].

The particularity in our case is the presence of liver metastasis in an asymptomatic patient, with a small-sized GIST which was later diagnosed, without the presence of central necrosis.

Median survival of patients with metastatic GIST is described in literature 51-57 months, and has radically increased after the emergence of tyrosine kinase inhibitors (TKIs), such as Imatinib mesylate treatment [17,18].

Conclusions

Hyperechoic liver lesions include a broad spectrum of pathologies, which most of the time require further investigations. GIST is a rare neoplasm of the gastrointestinal tract, difficult to diagnose because of its size, unusual location in the submucosal layer and lack of symptoms, that is rarely found in a metastatic stage.

The particularity in our case consists of the unusual presentation with the lack of specific symptoms and signs, and the fact that metastatic lesions were already present at the moment of the diagnosis of GIST.

Authors' contribution

AMR – Software, writing original draft, writing review and editing

DD – Project administration; Supervision; Validation)

CF – Formal analysis, writing review and editing

AB – Conceptualization, project administration, supervision, validation, writing review and editing

Conflict of interest

The authors have no conflict of interests.

Acknowledgment

A signed statement of informed consent to publish was signed by the patient (Nr. 6003/26.02.2018).

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CASE REPORT

Case Presentation: Unusual Association Between Possible Bilateral Intraventricular Xanthogranulomas, Postero-inferior Cerebellar Artery Aneurysm and Thrombophilia

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Introduction: Xanthogranulomas are rare, benign, usually asymptomatic, cutaneous tumors most frequently seen in children (juvenile xanthogranulomas). Some lesions can be found accidentally at randomly performed cerebral computer tomography (CT) or magnetic resonance imaging (MRI) or even on autopsy. **Case report**: We present the case of a 44 year-old woman, known with a thrombophilic disorder (PAI-1 gene mutation, MTHFR C677T and A1298C) on chronic anticoagulant treatment. The onset of symptoms was in 2010, when she presented paresthesia and lower limbs weakness. Two years later the patient presents with severe intermittent headache and left hemicrania and a cerebral angio-MRI is performed showing a left postero-inferior cerebellar artery aneurysm and two choroid plexus intraventricular masses in the lateral ventricles. The patient developed a new symptom, dysarthria in 2014 and in 2015 has multiple episodes of loss of consciousness, interpreted as epileptic seizures. Routine blood tests were within normal range, except for a high cholesterol level. The patient was tested for autoimmune, infectious, endocrine and metabolic diseases that were negative. Surgical treatment and biopsy from the lesion was proposed, however the patient refused both procedures. **Conclusions**: There is an association between xanthogranulomas localization and the choroid plexus, the most frequent CNS origin being in the trigon of the lateral ventricle. Our case does not resemble with any other case published, mostly because the unusual presentation, symptomatology and the association between xanthogranulomas, thrombophilia and postero-inferior cerebellar artery aneurysm which were never reported before in other cases of xanthogranulomas from the literature.

Keywords: xanthogranuloma, thrombophilia, cerebral aneurysm, choroid plexus

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Introduction

Xanthogranulomas (XG) are rare, benign tumors, being a common form of non-Langerhans cell histiocytosis, most frequently seen in children (juvenile XG) and in the majority of cases are asymptomatic. The most usual localization of XG is the skin, with a predilection for the neck and head region, but it can affect any organ, including the central nervous system (CNS). Some lesions can be found accidentally at randomly performed cerebral computer tomography (CT) or magnetic resonance imaging (MRI) or even on autopsy.

The most frequent cerebral localization for XG is the third ventricle, followed by the lateral ventricle and exceptionally the fourth ventricle.

The real incidence of this pathology is unknown, mostly because it is frequently asymptomatic or misdiagnosed as cystic degenerations of the choroid plexus, however according to some literature data, it was estimated at 1.6-7.0% of autopsies [1-3]

Case report

We present the case of a 44 year-old woman, known with a thrombophilic disorder (PAI-1 gene mutation, MTHFR C677T and A1298C) on chronic anticoagulant treatment The onset of the current symptomatology was in 2010, when she presented paresthesia and lower limbs weakness. A spine MRI was performed showing a dorsal arachnoid cyst for which she underwent surgery, the cyst being removed, however there was no significant improvement in her symptomatology. A year later, she also presented upper limbs weakness and a second spine MRI was performed, describing a posterior spinal cord atrophy without any signs of compression (Figure 1).

In 2012, the patient presents with severe intermittent headache and left hemicrania that responded partially positive to antalgics. Cerebral angio-MRI showed a left postero-inferior cerebellar artery aneurysm and two choroid plexus intraventricular masses in the lateral ventricles. The bilateral tumors were hypointense in T1 and hyperintense in T2 and FLAIR, and without gadolinium enhancement (Figure 2). Surgical clipping for the aneurysm was performed, with the alleviation of the algic symptoms, but without improvement of the motor deficit.

The patient developed a new symptom, dysarthria in 2014 and in 2015 has multiple episodes of loss of consciousness, preceded by intense generalized headache, with no response to antialgic treatment, hiccups and occasion-

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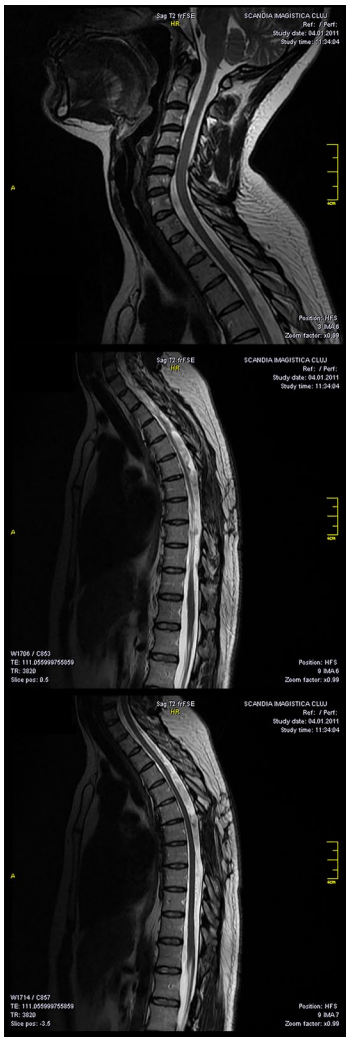

Fig. 1. Spine MRI – T2 sequence – Sagittal view

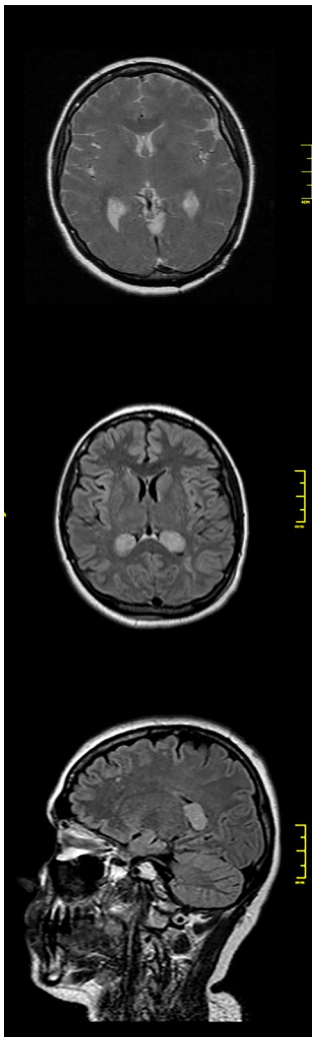

Fig. 2. Cerebral MRI – FLAIR sequence

ally vomiting with postictally aggravation of symptoms. This occurred once in two to three months with variable duration from 30 minutes up to 2-3 hours. This new symptoms were interpreted as generalized epileptic seizures. An electroencephalogram was performed, with no pathological changes.

Clinical examination revealed tetraparesis, predominantly affecting the lower limbs, ataxia, postural tremor, incoordination, brisk deep tendon reflexes, abolished abdominal reflexes, lower limbs hypoesthesia, right leg hypopalesthesia and dysarthria. No other pathological signs were found in the general examination of the patient.

In 2016 cerebral MRI was repeated showing multiple nonspecific subcortical demyelinating lesions, with no contrast enhancement.

Routine blood tests were within normal range, except for the cholesterol level which was slightly elevated. The patient was tested for autoimmune diseases (ANCA, anticardiolipin antibodies, C3, C4 complement level, ANA, Anti-dsDNA, cryoglobulins), infectious diseases (HIV, Cytomegalovirus, Toxocara canis, Ebstein Barr Virus, Lyme disease) endocrine and metabolic diseases (ceruloplasmin, plasma copper level, TSH, FT4) that were negative.

Surgical treatment and biopsy from the lesion was proposed, however the patient refused both procedures.

The patient continued anticoagulant treatment for the thrombophilic disorder and the preventive antiepileptic chronic treatment. The patient was referred to kinetotherapy and begun physical therapy.

Discussions

XG are benign tumors that occur throughout the body, however primary involvement of XG in the CNS is rare. [4,5]

Blumer described the first known case of choroid plexus XG in 1900, naming it cholestomatous endothelioma.

The prevalence of XG shows its peak between the 2nd and 4th decade of life and seems to have no sex predilection [6]. Shuangshoti et al published a small group of cases of XG (n=35) showing almost no difference between sex distribution (M:F=18:17) with more than 50% of the patients being between 20-50 years old [2]. In our case the prevalence described in the literature corresponds with the patient's profile, being a 44-year-old female.

It is not clearly understood if XG is associated with other pathologies, but in several isolated case reports there was suggested extracutaneous involvement alongside the breast [7], eye [8] and CNS [6]. In our case the patient presented an unusual association with a postero-inferior cerebellar artery aneurysm and thrombophilia, none of these pathologies being previously reported with XG. There are also no genetic mutations related with XG in the literature, even if in our case the patient had PAI-1, MTHFR, C677T and A1298C gene mutations confirmed.

The association between XG and choroid plexus has been stated in several case reports, being the most common origin point for XG masses in CNS. [9-12]

The most frequent localization of XG in CNS is in the trigon of the lateral ventricle, a less common location being the third ventricle [13,14].

XG usually remains asymptomatic, because the size of the masses is too small to obstruct the cerebrospinal fluid (CSF) flow. They become symptomatic when they have increased in size enough in order to be clinically significant by obstructing CSF flow, the most frequent symptomatic XG being located in the third ventricle where they obstruct the foramen of Monro resulting in hydrocephalus[15].

Histopatologically, XG masses consists of dense infiltrates of histiocytes with several multinucleated giant cells along with eosinophils lymphocytes and plasma cells that are often found. Cells from the altered epithelium of the choroid plexus become detached and disintegrate, releasing lipid into the choroidal matrix which will result in a xanthogranuloma. XG usually consists of cholesterol crystals, giant cells, foci of hemorrhage alongside proliferation of small blood vessels and granules of hemosiderin resulted in the process. XG pathogenesis is still debatable, several theories were issued regarding the occurrence and etiology of the disease. Some authors proposed a tissue reaction to

hemorrhage, others suggested a disturbance in lipids metabolism found in familial hypercholesterolemia and proliferation of different type of cells, while other authors like Wolf and Ayres described the "foamy cells" derived from arachnoid cells in the choroid stroma and proliferation of epithelial cells of the choroid plexus [16]. Razavi-Encha studied intraventricular lesions with electronic microscopy and noticed some leptomeningeal cells at the origin of xanthogranulomas[9].The epithelium of the choroid plexus presents cellular stratification and height reduction as they age, some of them becoming squamous. They may desquamate and increase in size becoming foamy by gathering intracytoplasmatic lipids P. Miranda et al analysed published cases until 2005 and noted fifteen cases of lateral ventricle choroid plexus xanthogranulomas and sixteen cases localized in the third ventricle, described histopathologic and radiologic findings in third ventricle masses [8]. Shuangshoti, et al resumed 35 cases of intraventricular xanthogranulomas analyzing the origin of foamy cells, symptomatology, imagery and postoperative course and stated that these intraventricular masses lead to increased intracranial pressure [2].

Chen et al performed laboratory tests and concluded a correlation between high traces of cholesterol and predisposing state in formation of xanthogranulomas of choroid plexus [17].

Regarding frequency and significance, the symptomatology encountered was: headache, nausea and vomiting, seizures, ataxia, nystagmus, double vision, hemiparesis, bladder incontinence, deterioration of consciousness, changes of personality and other symptoms secondary to intracranial hypertension [15,18]. In our case, the patient had a progressive symptomatology over the course of several years, which could not be explained by other associated pathologies, as there was no improvement after the arachnoid cyst was removed and the posterior spinal cord atrophy could explain the paresthesias, but not the limb weakness.

Deterioration of consciousness was described as a result of compression of cerebral structures and brainstem by the herniation of hippocampal gyrus or the cerebral tonsils that could lead to death [18]. In our case, the loss of consciousness was interpreted in the context of epileptic seizures, which could have been triggered by intracranial hypertension secondary to XG.

Radiologic findings showed intraventricular masses localized within the glomus of choroid plexus, in both trigones of the lateral ventricles or in the third or fourth ventricle.

In the CT scan studies, XG showed different densities in comparison to brain tissue, both hypodense and hyperdense lesions being described. When large in size, XG cannot be distinguished from other lesions such as degenerated cystic glomera by CT scan [19].

MRI performed showed in several cases hyperintense signal in T2, explained by the high solid lipidic components of XG, and also iso or hyperintense signal in T1 sequences [20].

The demyelinating lesions described in our patient's second MRI are not specific for XG and the suspicion of cerebral vasculitis was risen. In order to confirm the diagnosis, a series of tests for autoimmune, infectious and metabolic disorders were performed, which led to the exclusion of the diagnosis.

Gadolinium enhancement modifications were reported in a small number of cases, without being specific for this kind of lesion. Also, Tc^{99} scans did not show abnormal uptake [21].

Xanthogranulomas are difficult to distinguish from choroid glomerular cysts, acute infarction of the choroid plexus, papilloma, meningioma, metastatic lesions, choroid plexus carcinoma and arteriovenous malformation. Radiological differential diagnosis should be made especially with choroid cysts which have altered signal due to protein and blood products and usually follow CSF on all sequences and acute infarction of the choroid plexus, which are usually located unilateral while XG is bilateral, both having high signal on DWI [21-23]. In order to put a definitive XG diagnosis, histopathology examination is required, however in our case biopsy was proposed, but the patient refused.

The treatment is necessary only in symptomatic patients and first-line treatment is surgical intervention with mass removal. Case-reports showed that post-surgery outcome was good in unilateral XG, complications such as visual loss or death being described in patients with bilateral lateral ventricle lesions. Poor outcome was encountered in XG located in the third and forth ventricle, with a large number of postoperative complications. [24,25].

Conclusion

XG is a rare benign disease, usually found accidentally as most of the cases are asymptomatic.

There is an association between XG localization and the choroid plexus, the most frequent CNS origin being in the trigon of the lateral ventricle.

Surgery remains the standard of treatment for XG, but should only be performed in symptomatic patients, because of the associated complications that could develop after the mass removal.

Our case does not resemble with any other case published, mostly because the unusual presentation, symptomatology and the associated pathologies which were never reported before in other cases of XG from the literature.

Authors'contribution

SRA – Software, writing original draft, writing review and editing
AR – Formal analysis, validation
ZB – Formal analysis, resources, supervision, visualization
SM – Data curation, investigation
LB – Methodology, project administration, visualization
RB – Conceptualization, supervision, validation, writing review and editing

Conflict of interest

The authors have no conflict of interests.

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A signed statement of informed consent to publish was signed by the patient (Nr. 18131/30.05.2016).

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