EDITOR-IN-CHIEF'S WORD

The guest of this issue of the Bulletin of the Croatian Academy of Engineering is our honorary member and prominent scientist in the field of personalized medicine, Prof. Dragan Primorac, M.M., Ph.D.

We asked him to briefly outline the latest achievements in this field and in particular through the activities of the Croatian Specialty Hospital Sv. Katarina, with which the Academy has a cooperation agreement and which was co-founded by Prof. Primorac.

I believe that you will read this article with interest, which is topical today and especially in times of general crisis caused socially by the COVID 19 pandemic.

Editor-in-Chief
Vladimir Andročec, President of the Croatian Academy of Engineering

EDITOR'S WORD

Dear readers,

It is my pleasure to present in this edition of the HATZ Bulletin the Engineering Power research activities being conducted at St Catherine Specialty Hospital in collaboration with their partner institutions.

The presented papers discuss technologically advanced clinical treatments that emphasize the personalized medicine approach in different important fields of contemporary clinical practice as well as their work related to COVID-19 pandemic issues.

The guest editor of this edition is Prof. Dragan Primorac, Ph.D. Honorary Member of the Croatian Academy of Engineering and professor at several academic institutions in Croatia, USA, China, India and Germany.

Editor
Zdravko Terze, Vice-President of the Croatian Academy of Engineering

FOREWORD

St. Catherine Specialty Hospital is amongst the first healthcare institutions that fully live the concept of personalized medicine. In our work, we are guided by our commitment to excellence in all segments of work, knowledge, collaboration with the best, systematic staff training, and the application of cutting-edge medical procedures and the latest scientific discoveries. Our highly trained medical experts and their unique work performance is our special strength. That's what sets us apart. We've intentionally set high standards, and we take all the necessary steps to reach them. Patient satisfaction is our only success indicator. The path of modern medicine is a combination of science and clinical medicine. This concept is called translational medicine. It's a great privilege to be part of a team that contributes to the development of science, which will ultimately impact human health. The concept of personalized or precision medicine is based on knowing and understanding processes on the molecular level, which is crucial to treatment. In other words, genome analysis provides information that may be important for disease prevention, making an early diagnosis, and finding optimal treatment, and monitoring the effectiveness of therapy. The right therapy for the right patient at the right time is the key motto of personalized medicine and is particularly important when it comes to pharmacogenomics. What we're particularly interested in our institution, is osteoarthritis therapy, a degenerative joint disease which is mainly caused by cartilage loss in the joints. It is assumed that more than 750 million people worldwide suffer from osteoarthritis. Osteoarthritis treatment has long been based solely on the modulation of pain and, in the most severe cases, implantation of a partial or total endoprosthesis. Great advances in the fields of tissue engineering and regenerative medicine have been made based on new findings. One of the methods is the use of mesenchymal stem cells in the treatment of osteoarthritis. We've achieved exceptional results best testified daily by our patients.

The following papers are part of the scientific research conducted in St. Catherine Specialty Hospital in collaboration with our partner institutions. They are a compilation of our previous work on these topics and we encourage the readers to read these papers in full, they are referenced at the end of each paper in this series.

Mesenchymal stem cells, autologous immunomodulatory effector cells, are discussed in the first paper in the context of orthopedics, where they have proven to be a new tool that can help patients who are not yet candidates for total joint replacement surgery. The individual approach we commit to in St. Catherine Specialty Hospital is stressed throughout the second paper, where we present a case of a patient suffering from osteogenesis imperfecta. The third paper is a short overview of pharmacogenomics' core concepts, a new field in clinical practice that combines molecular genetic analysis with actionable therapeutic interventions that are unique for each patient. Finally, the fourth paper addresses the pandemic of COVID-19, which has stopped our lives in a place in the past year. We decided to present our work on the effect of environmental factors on SARS-CoV-2 and IgG glycome composition in COVID-19 patients.

I hope you will find our work interesting and encourage you to contact us if you have any questions or comments; we would be happy to hear from you.

Guest-Editor
Professor Dragan Primorac, M.D., Ph.D.

CONTENT

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Mesenchymal stem cells in orthopedics - a current perspective

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Abstract

Osteoarthritis is a common condition that can affect any joint in the body. It is encountered in all age groups, but with a higher incidence in the older population. There is no treatment currently available that would prevent the development or progression of osteoarthritis and the gold standard end-stage treatment is still total joint replacement surgery, which is not without its risks. Therefore, new approaches are considered daily to treat patients that are not yet at end-stage osteoarthritis, but still experience the most common symptoms of pain and joint dysfunction. Mesenchymal stem cell research offers new opportunities for osteoarthritis treatment as their paracrine effect exhibits clinical improvement in osteoarthritis patients, providing much-needed minimally invasive treatment options.

Keywords: osteoarthritis, regenerative medicine, MSCs, lipoaspiration

Introduction

Osteoarthritis (OA) is a progressive degenerative condition that can affect any joint in the body, but it primarily affects the knees, hips and hand joints [1-3]. The economic burden of OA is at least $89.1 billion annually [4,5]. Years of research in OA pathophysiology resulted in our better understanding of the underlying processes, as OA is now recognized as a whole joint disease that affects articular cartilage and subchondral bone, Hoffa's fat pad, synovia, ligaments, and muscles (Figure 1) [6-8]. The dominant symptoms of OA are joint pain and reduced motion that can be treated either pharmacologically or surgically, with total joint replacement surgery as an end-stage treatment [9].

OA affects 40% of people older than 70, presenting with first symptoms at the age of 55, suggesting that patients are living with decreased mobility and pain for more than 20 years [10,11]. It is approximated that 250 million people suffer from OA worldwide, with the female sex being at a higher risk of developing OA than men [12,13]. The observed difference in sex distribution can be attributed to different female anatomy compared to the one in males, such as narrower femurs, thinner patellae, larger angles of quadriceps and differences in the size of tibial condyles; leading to different kinematics and making women more likely to develop OA [14]. Obesity, increased body mass index (BMI), previous knee injury or malalignment are strong risk factors for knee OA, whereas hip deformities play a great role in
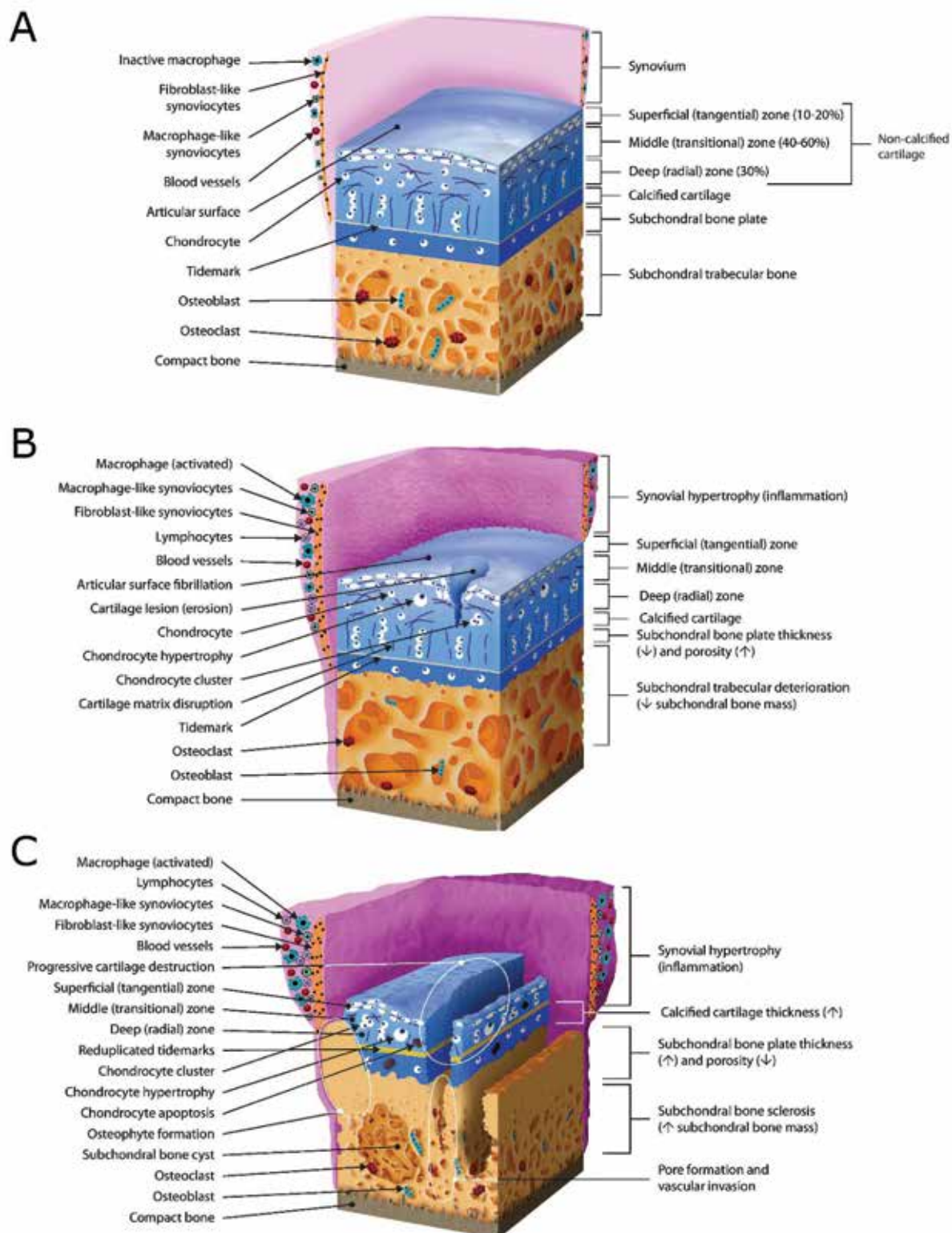
Figure 1. Microarchitectural and histologic changes of articular cartilage, subchondral bone and synovium in osteoarthritis.
(A) Normal joint structure; (B) Early-stage osteoarthritis; (C) Late-stage osteoarthritis.

developing hip OA [15]. Any repetitive action that causes frequent injuries and/or cartilage defects such as: often kneeling, heavy lifting and professional sports activities are associated with higher risk of developing OA [12]. Additionally, the previous injury of ligament structures such as meniscal and anterior cruciate ligament tears also increases the risk of OA development [16]. One the other hand, physical inactivity causes higher susceptibility to joint damage, due to less stable and weaker joints [16]. Some studies indicate there is a connection between OA and a slightly increased risk of developing the cardiovascular and atherosclerosis-related disease [12,17].

Patients with lower limb OA are also more likely to suffer from chronic pain, causing a cycle in which pain limits physical activity and physical inactivity contributes to greater knee pain and weight gain, potentially resulting in depressive symptoms as a consequence of OA [15,16]. Therapeutic measures used in treating OA symptoms include both pharmacological and non-pharmacological methods, the choice of which is dependent on disease stage, patient characteristics and comorbidities. Pharmacological treatment includes oral, topical and intraarticularly used analgesics, anti-inflammatory drugs or other substances. Lately, biological therapies such as platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs) became widely used in treating patients with OA, even though the leading guidelines advise against their use due to lack of high-quality studies or a straight clinical protocol [18,19] These new agents may slow down the existing condition, alleviate OA symptoms and postpone the need of surgery.

Mesenchymal stem cells

In recent years, due to the increasing use of Mesenchymal stem cells (MSCs) in clinical practice around the world, research on MSCs has become increasingly extensive and relevant. MSCs are adult stem cells present in various tissues throughout the body. They have the potential to differentiate into various cell types and secrete immunomodulatory and trophic signaling

molecules that promote local regeneration by secretion of anti-apoptotic, anti-scarring, angiogenic and mitotic signaling molecules, and inhibit bacterial growth by secreting LL-37 [20] (Figure 2). These immunomodulatory and paracrine mechanisms are responsible for their clinical effect, putting them in the focus of regenerative medicine for OA and other medical conditions (Figure 1). The main effects of MSC therapy on knee OA is pain reduction and mobility improvement measured by visual analog scale (VAS), Western Ontario and McMaster Universities Arthritis Index (WOMAC) and Knee Injury and Osteoarthritis Outcome Score (KOOS), while the reported effect on the articular cartilage has not been constant, with studies reporting various end effects regarding both volume and structure, measured by MRI or second-look arthroscopies [21-26]. When considering MSC therapy, factors such as the amount of harvest volume, cell isolation procedure, isolated cell number, regenerative capacity of certain cells and the side effects of therapy have to be assessed, in order to determine the best harvest site for MSCs [27]. Still, key problems associated with MSC therapy include dosing, harvest site, and the number of delivered MSCs, as there is no standard procedure that can answer these questions. Typical harvest sites for MSCs are the bone marrow and adipose tissue. Other sites include the umbilical cord and the placenta [28,29]. The safety of MSCs in the treatment of various musculoskeletal pathologies has been thoroughly studied and confirmed [28,30].
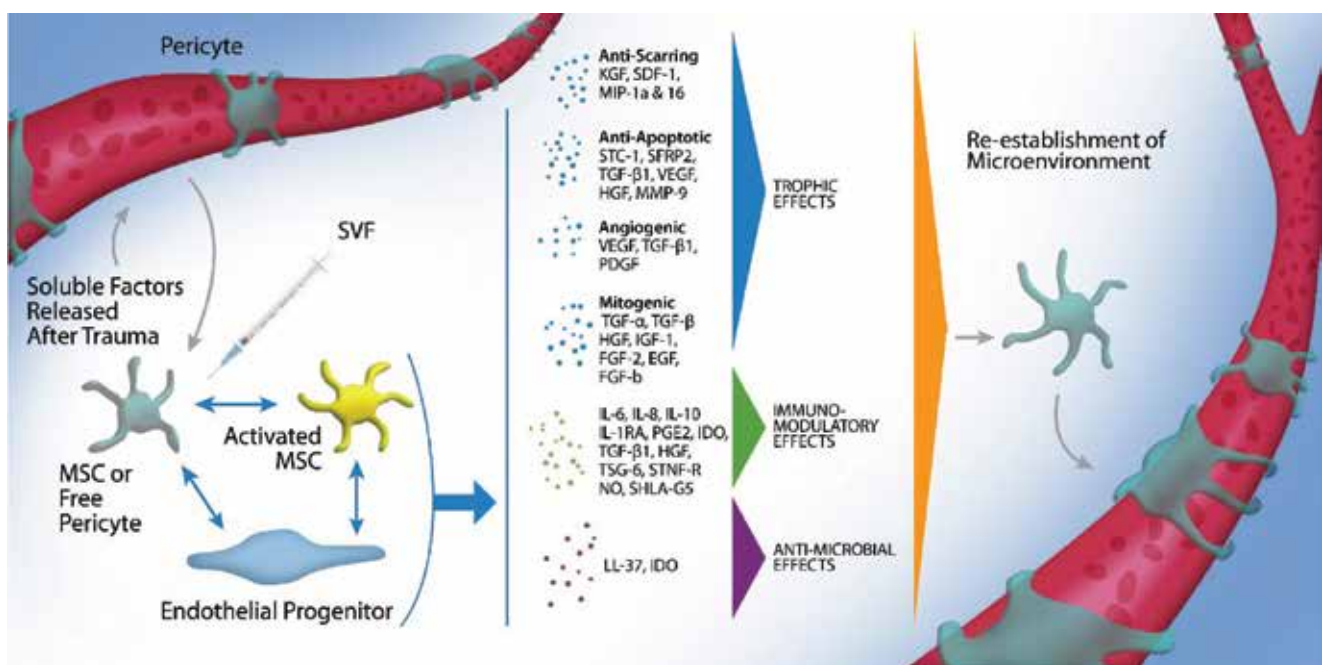

Figure 2. The trophic, immunomodulatory and antimicrobial effects of mesenchymal stem cells or medicinal signaling cells (MSCs). Pericytes are stimulated by soluble growth factors and chemokines to become activated MSCs, and probably in interaction with endothelial progenitors both cell types respond to the microenvironment by secreting trophic (mitogenic, angiogenic, anti-apoptotic, or scar reduction), immunomodulatory or antimicrobial factors. After the microenvironment is re-established, MSCs return to their native pericyte state attached to blood vessels. SVF – stromal vascular fraction. Murphy BM, Moncivais K and Caplan IA. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Experimental and Molecular Medicine (2013) 45, e54; doi:10.1038/emm.2013.94 (Used and adapted with permission of Prof. Arnold I Caplan and publisher's permission).

Bone-marrow mesenchymal stem cells (BM-MSCs)s

BM-MSCs are usually obtained by aspiration from the posterior or anterior iliac crest. Density-gradient centrifugation of the aspirate is needed to produce a bone marrow aspirate concentrate (BMAC), during which the number of MSCs and growth factor containing platelets is increased [31,32]. The application is performed by an intra-articular injection into the target joint. Clinical results of BM-MSCs therapy are generally positive. As previously mentioned, significant improvements in pain levels and function levels were observed in a meta-analysis when compared to VAS, KOOS, WOMAC and Lysholm scores prior to the procedure [33]. In a literature review of research published between 2014 and 2019 an association of cell count and treatment outcomes was observed. In individuals with grade ≥ 2 knee OA on the Kellgren-Lawrence scale, a moderate-high number of cells (40×106) was found to achieve an optimal effect, while lower (24×106) and higher (100×106) cell numbers, were associated with an increase in observed adverse effects, such as persistent knee pain and swelling [34].

Adipose-derived mesenchymal stem cells (AD-MSCs)

Usually obtained from subcutaneous adipose tissue by lipoaspiration, these procedures are less invasive compared to BM-MSCs extraction [35]. Adipose tissue provides a significant, easily accessible source of cells contained in stromal vascular fraction (SVF) and provides a large number of cells from which multipotent AD-MSCs can be isolated, containing 500 times more MSCs compared to the same volume of bone marrow [27,36,37]. As stated previously, the various procedures of MSC harvesting, processing and application are the key limitations of their introduction to standard daily clinical practice and guidelines of professional societies. Therefore, we analyzed the cell populations in the stromal vascular fraction from lipoaspirate (SVF-LA) and stromal vascular fraction from microfragmented lipoaspirate (SVF-MLA). We identified the following cell phenotypes: endothelial progenitor cells (EPC), endothelial mature cells, pericytes, transitional pericytes, and supra adventitial-adipose stromal cells (SA-ASC) (Figure 3). Compared to SVF-LA, SVF-MLA was dominated by a reduction of leukocytes and SA-ASC, and an increase of EPC, suggesting their enrichment by the process of micro-fragmentation, thus indicating their role in the observed effect of MSC therapy on knee OA [38].

Our results

In our institution, we investigated the use of autologous microfragmented adipose tissue (AMFAT) in the treatment of knee osteoarthritis. The effect of intraarticular
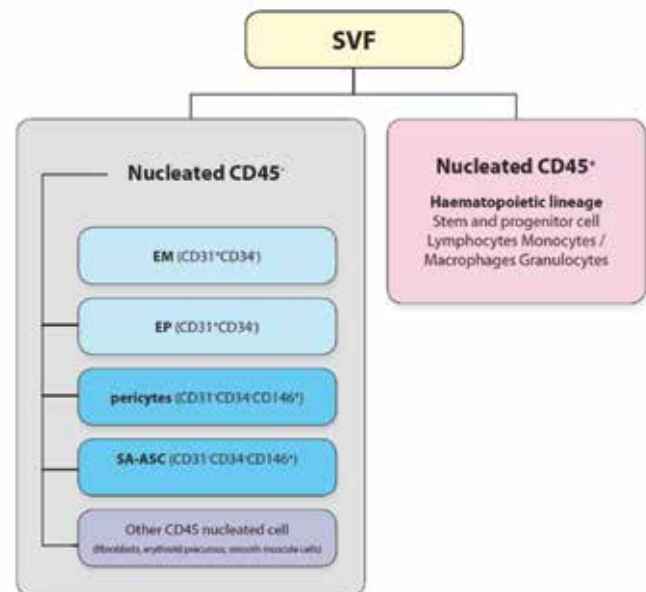

Figure 3. Summarized results of main immunophenotypes in the stromal vascular fraction (SVF) nucleated cell populations from microfragmented adipose tissue isolated from 20 osteoarthritic patients. EMC – endothelial mature cells, EPC – endothelial progenitor cells, SA-ASC – supra-adventitial adipose stromal cells.

injection of AMFAT on a series of 17 patients with late-stage knee OA (Kellgren-Lawrence grades III and IV) was studied, using quantitative MRI assessment of glycosaminoglycan (GAG) content in cartilage by using delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC) index, as well as clinically by a standard orthopedic physical examination and VAS assessment. A standard lipoaspiration technique was performed, and the harvested fat was micro-fragmented and applied intraarticularly into the patients' affected knee joints. Pain estimates measured by VAS decreased significantly, both for resting and movement estimates at 3, 6 and 12-month follow-ups. Cartilage GAG content, measured by delayed gadolinium-enhanced magnetic resonance imaging of cartilage dGEMRIC index, significantly improved in 52.9% of measurements and deteriorated in only 11.2% of measurements, which would be a normal disease course for the late-stage OA [39]. In our second study, functional scores were assessed at 12 months follow-up for 20 patients. Seventeen patients (85%) showed a significant improvement in KOOS and WOMAC scores. KOOS improved from 46 to 176% when compared with baseline, WOMAC increased from 40 to 45%, while VAS rating increased from 54% to 82%. Three patients (15%) were subjected to total knee replacement surgery and were excluded from the study [40]. The last trial included ten patients (18 knees) suffering from knee OA grades III and IV who were assessed for GAG content and clinical outcome after a single intraarticular injection of AMFAT in a 2-year period. Study results indicated an improvement of GAG content, measured by the dGEMRIC index, with more than half of the measurements signifying a relevant

improvement in a 2-year follow-up, which is in contrast to GAG content reduction over the natural course of the disease. VAS pain score also significantly decreased over the 24-month period, both in resting and movement [41]. Taken together, these studies suggest that the application of autologous microfragmented adipose tissue with SVF in patients with knee OA increases GAG levels in hyaline cartilage, consequently reducing pain and improving movement abilities, while also postponing the need for total joint replacement surgery in patients with more advanced OA stage.

Conclusions

Despite the current negative stance of the official guidelines on MSC treatment, the minimally invasive, one-step, economic procedure of their application and positive patient outcomes indicated both by pain reduction and increased GAG content cannot be neglected. Promising results and rare adverse events encourage future studies that would determine exact dosing, harvest site, and the number of delivered MSCs in a standard procedure. While joint replacement surgery still represents the gold standard in the treatment of OA, MSCs therapy provides a possibly great alternative and it is assumed that it will take a major role in future OA treatment, especially in patients that are not yet candidates for joint replacement surgery.

Note: Mesenchymal stem cells in orthopedics - current perspective is an excerpt from the texts prepared for: Genes, scientific journal (Primorac D, Molnar V, Rod E, Jeleč Ž, Čukelj F, Matišić V, Vrdoljak T, Hudetz D, Hajsok H, Borić I. Knee Osteoarthritis: A Review of Pathogenesis and State-Of-The-Art Non-Operative Therapeutic Considerations. Genes (Basel). 2020 Jul 26; 11(8):854. doi: 10.3390/genes11080854.), Journal of Stem Cells Research, Development & Therapy (Perić V, Kottek T, Molnar V, Matišić V, Čukelj F, Primorac D. Mesenchymal Stem Cells in the Treatment of Knee Osteoarthritis. J Stem Cell Res Dev Ther 6: 050.) and Croatian Medical Journal (Hudetz D, Borić I, Rod E, Jeleč Ž, Kunovac B, Polašek O, Vrdoljak T, Plečko M, Skelin A, Polančec D, Zenić L, Primorac D., Early results of intra-articular microfragmented lipoaspirate treatment in patients with late stages knee osteoarthritis: a prospective study. Croat Med J. 2019 Jun 13;60(3):227-236. doi: 10.3325/cmj.2019.60.227.) in which the comprehensive review of osteoarthritis pathophysiology and MSC treatment were prepared and presented in full.

References

[1] Bortoluzzi, A., Furini, F., Scirè, C.A. Osteoarthritis and its management - Epidemiology, nutritional aspects and environmental factors [Internet]. Vol. 17, Autoimmunity Reviews. Elsevier B.V.; 2018 [cited 2020 Apr 15]. p. 1097–104. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1568997218302088

[2] Mabey, T., Honsawek, S. Cytokines as biochemical markers for knee osteoarthritis. World J Orthop. 2015;6(1):95–105.

[3] Nelson, A.E. Osteoarthritis year in review 2017: clinical [Internet]. Vol. 26, Osteoarthritis and Cartilage. W.B. Saunders Ltd; 2018 [cited 2020 Apr 15]. p. 319–25. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1063458417313481

[4] Carlson, A.K., Rawle, R.A., Wallace, C.W., Brooks, E.G., Adams, E., Greenwood, M.C., Olmer, M., et al. Characterization of synovial fluid metabolomic phenotypes of cartilage morphological changes associated with osteoarthritis. Osteoarthr Cartil. 2019;27(8):1174–84.

[5] Vos, T., Abajobir, A.A., Abbafati, C., Abbas, K.M., Abate, K.H., Abd-Allah, F., Rizwan, S.A., et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1211–59.

[6] Ilas, D.C., Churchman, S.M., McGonagle, D., Jones, E. Targeting subchondral bone mesenchymal stem cell activities for intrinsic joint repair in osteoarthritis. Futur Sci OA. 2017;3(4).

[7] Swingler, T.E., Niu, L., Smith, P., Paddy, P., Le, L., Barter, M.J., Young, D.A., et al,. The function of microRNAs in cartilage and osteoarthritis. Clin Exp Rheumatol. 2019;37(5):40–7.

[8] Martel-Pelletier, J., Barr, A.J., Cicuttini, F.M., Conaghan, P.G., Cooper, C., Goldring, M.B., Goldring, S.R., Jones, G., et al. [Internet]. Vol. 2, Nature Reviews Disease Primers. Nature Publishing Group; 2016 [cited 2020 Apr 3]. p. 16072. Available from: http://www.nature.com/articles/nrdp201672

[9] Primorac, D., Molnar, V., Rod, E., Jeleč, Ž., Čukelj, F., Matišić, V., Vrdoljak, T., et al. Knee Osteoarthritis: A Review of Pathogenesis and State-Of-The-Art Non-Operative Therapeutic Considerations. Genes (Basel). 2020 Jul 26;11(8):854.

[10] Van Spil, W.E., Kubassova, O., Boesen, M., Bay-Jensen, A.C., Mobasheri, A. Osteoarthritis phenotypes and novel therapeutic targets. Biochem Pharmacol. 2019;165:41–8.

[11] Tachmazidou, I., Hatzikotoulas, K., Southam, L., Esparza-Gordillo, J., Haberland, V., Zheng, J., Johnson, T., et al. Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK Biobank data. Nat Genet. 2019;51(2):230–6.

[12] Hunter, D.J., Bierma-Zeinstra, S. Osteoarthritis. Lancet. 2019;393(10182):1745–59.

[13] Carlson, A.K., Rawle, R.A., Wallace, C.W., Brooks, E.G., Adams, E., Greenwood, M.C., Olmer, M., et al. Characterization of synovial fluid metabolomic phenotypes of cartilage morphological changes associated with osteoarthritis. Osteoarthr Cartil. 2019;27(8):1174–84.

[14] Hame, S.L., Alexander, R.A. Knee osteoarthritis in women. Curr Rev Musculoskelet Med. 2013;6(2):182-7.

[15] Vina, E.R., Kwoh, C.K. Epidemiology of osteoarthritis: Literature update. Curr Opin Rheumatol. 2018;30(2):160–7.

[16] Berenbaum, F., Wallace, I.J., Lieberman, D.E., Felson, D.T. Modern-day environmental factors in the pathogenesis of osteoarthritis Nature Reviews Rheumatology. 2018;14:674–81.

[17] Wallace, I.J., Worthington, S., Felson, D.T., Jurmain, R.D., Wren, K.T., Maijanen, H., Woods, R.J., Lieberman, D.E. Knee osteoarthritis has doubled in prevalence since the mid-20th century. Proc Natl Acad Sci U S A. 2017;114(35):9332–6.

[18] Bannuru, R.R.;,Osani, M.C., Vaysbrot, E.E., Arden, N.K., Bennell, K., Bierma-Zeinstra, S.M.A., Kraus, V.B.; et al. OARSI Guidelines for the Non-Surgical Management of Knee, Hip, and Polyarticular Osteoarthritis. Osteoarthr Cartil. 2019;27:1578–1589.

[19] Kolasinski, S.L., Neogi, T., Hochberg, M.C., Oatis, C., Guyatt, G., Block, J., Callahan, L., et al. 2019 American College of Rheumatology/Arthritis Foundation Guideline for the Management of Osteoarthritis of the Hand, Hip, and Knee. Arthritis Care Res. 2020;72:149–162.

[20] Caplan, A.I., Hariri, R. Body Management: Mesenchymal Stem Cells Control the Internal Regenerator. Stem Cells Transl Med. 2015;4:695–701.

[21] Kim, S.H., Ha, C.W., Park, Y.B., Nam, E., Lee, J.E., Lee, H.J. Intra-articular injection of mesenchymal stem cells for clinical outcomes and cartilage repair in osteoarthritis of the knee: a meta-analysis of randomized controlled trials. Vol. 139, Archives of Orthopaedic and Trauma Surgery. Springer Verlag; 2019. p. 971–80.

[22] Kim, S.H., Djaja, Y.P., Park, Y.B., Park, J.G., Ko, Y.B., Ha, C.W. Intra-articular Injection of Culture-Expanded Mesenchymal Stem Cells Without Adjuvant Surgery in Knee Osteoarthritis: A Systematic Review and Meta-analysis. Am J Sports Med. 2020;48(11):2839–49.

[23] Ma, W., Liu, C., Wang, S., Xu, H., Sun, H., Fan, X. Efficacy and safety of intra-articular injection of mesenchymal stem cells in the treatment of knee osteoarthritis: A systematic review and meta-analysis. Medicine (Baltimore). 2020;99(49):e23343.

[24] Song, Y., Zhang, J., Xu, H., Lin, Z., Chang, H., Liu, W., Kong, L. Mesenchymal stem cells in knee osteoarthritis treatment: A systematic review and meta-analysis. J Orthopaedic Translation. 2020;24: 121–30.

[25] Maheshwer, B., Polce, E.M., Paul, K., Williams, B.T., Wolfson, T.S., Yanke, A., Verma, N.N., et al. Regenerative Potential of Mesenchymal Stem Cells for the Treatment of Knee Osteoarthritis and Chondral Defects: A Systematic Review and Meta-analysis. Arthroscopy. 2021;37:362–78.

[26] Ha, C.W., Park, Y.B., Kim, S.H., Lee, H.J. Intra-articular Mesenchymal Stem Cells in Osteoarthritis of the Knee: A Systematic Review of Clinical Outcomes and Evidence of Cartilage Repair. Arthroscopy. 2019;35:277-288.e2.

[27] Shariatzadeh, M., Song, J., Wilson, S.L. The efficacy of different sources of mesenchymal stem cells for the treatment of knee osteoarthritis. Cell Tissue Res. 2019;378:399–410.

[28] McIntyre, J.A., Jones, I.A., Danilkovich, A., Vangsness, C.T. The Placenta: Applications in Orthopaedic Sports Medicine. Am J Sports Med. 2018;46:234–47.

[29] Kern, S., Eichler, H., Stoeve, J., Klüter, H., Bieback, K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells. 2006;24(5):1294-301.

[30] Di Matteo, B., Vandenbulcke, F., Vitale, N.D., Iacono, F., Ashmore, K., Marcacci, M., Kon, E. Minimally Manipulated Mesenchymal Stem Cells for the Treatment of Knee Osteoarthritis: A Systematic Review of Clinical Evidence. Stem Cells Int. 2019, 2019, 1–14.

[31] Kasten, P., Beyen, I., Egermann, M., Suda, A., Moghaddam, A., Zimmermann, G., Luginbühl, R. Instant stem cell therapy: Characterization and concentration of human mesenchymal stem cells in vitro. Eur Cells Mater. 2008;16:47–55.

[32] Madry, H., Gao, L., Eichler, H., Orth, P., Cucchiarini, M. Bone Marrow Aspirate Concentrate-Enhanced Marrow Stimulation of Chondral Defects. Stem Cells Int. 2017;2017:1–13.

[33] Awad, M.E., Hussein, K.A., Helwa, I., Abdelsamid, M.F., Aguilar-Perez, A., Mohsen, I., Hunter, M., et al. Meta-Analysis and Evidence Base for the Efficacy of Autologous Bone Marrow Mesenchymal Stem Cells in Knee Cartilage Repair: Methodological Guidelines and Quality Assessment. Stem Cells Int. 2019;2019:1–15.

[34] Doyle, E.C., Wragg, N.M., Wilson, S.L. Intraarticular injection of bone marrow-derived mesenchymal stem cells enhances regeneration in knee osteoarthritis. Knee Surgery, Sport Traumatol Arthrosc. 2020; 28(12):3827-3842,

[35] Lu, L., Dai, C., Zhang, Z., Du, H., Li, S., Ye, P., Fu, Q., et al. Treatment of knee osteoarthritis with intra-articular injection of autologous adipose-derived mesenchymal progenitor cells: a prospective, randomized, double-blind, active-controlled, phase IIb clinical trial. Stem Cell Res Ther. 2019;10:143.

[36] Damia, E., Chicharro, D., Lopez, S., Cuervo, B., Rubio, M., Sopena, J., Vilar, J.M., et al. Adipose-Derived Mesenchymal Stem Cells: Are They a Good Therapeutic Strategy for Osteoarthritis? Int J Mol Sci. 2018;19:1926.

[37] Bora, P., Majumdar, A.S. Adipose tissue-derived stromal vascular fraction in regenerative medicine: a brief review on biology and translation. Stem Cell Res Ther. 2017;8:145.

[38] Polančec, D., Zenić, L., Hudetz, D., Borić, I., Jeleč, Ž., Rod, E., Vrdoljak, T., et al. Immunophenotyping of a Stromal Vascular Fraction from Microfragmented Lipoaspirate Used in Osteoarthritis Cartilage Treatment and Its Lipoaspirate Counterpart. Genes (Basel). 2019;10:474.

[39] Hudetz, D., Borić, I., Rod, E., Jeleč, Ž., Radić, A., Vrdoljak, T., Skelin, A., et al. The Effect of Intra-articular Injection of Autologous Microfragmented Fat Tissue on Proteoglycan Synthesis in Patients with Knee Osteoarthritis. Genes (Basel). 2017;8:270.

[40] Hudetz, D., Borić, I., Rod, E., Jeleč, Ž., Kunovac, B., Polašek, O., Vrdoljak, T., et al. Early results of intra-articular micro-fragmented lipoaspirate treatment in patients with late stages knee osteoarthritis: a prospective study. Croat Med. J. 2019;60:227–236.

[41] Borić, I., Hudetz, D., Rod, E., Jeleč, Ž., Vrdoljak, T., Skelin, A., Polašek, O., et al. A 24-Month Follow-Up Study of the Effect of Intra-Articular Injection of Autologous Microfragmented Fat Tissue on Proteoglycan Synthesis in Patients with Knee Osteoarthritis. Genes (Basel). 2019;10:1051.

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Osteogenesis imperfecta type III – a short review and an example of personalized surgery approach

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Abstract

Osteogenesis imperfecta (OI) or brittle bone disease, a heritable disorder of connective tissue, is the most common of the inherited disorders primarily affecting bone. There are approximately 400 individuals with OI in Croatia alone. The basis of this disease in European populations is mostly the result of defects in the structure or processing of collagen type I, an important protein of the extracellular matrix in many tissues. Although fractures occurring with no injury or minor injury are the hallmark of OI, other non-mineralized tissues can be affected as well. Four different types of the disease are commonly distinguished, ranging from a mild condition (type I) to a lethal one (type II). Types III and IV patients present with severe forms. Due to the relatively low prevalence in the general population, treating physicians have limited experience with this disease, both with children or adults. As an example of personalized surgery approach, we present an 11-year-old patient with OI type III. Before referral to our hospital, she was treated with 18 cycles of bisphosphonates as well as with several different surgical procedures. The patient underwent two surgeries at our hospital with a 5-month interval between them. Using the Fassier-Duval intramedullary telescoping nail, correction osteotomies of both femurs and lower legs in two separate settings were performed, with a very good final result.

Keywords: osteogenesis imperfecta, orthopedics, pediatrics, personalized medicine

Introduction

Osteogenesis imperfecta (OI) or brittle bone disease is a genetic disorder with varying clinical forms, from those lethal at birth, to mild ones that phenotypically resemble postmenopausal osteoporosis. The disease occurs at a frequency of 6–7/100 000 newborns [1]. Although fractures occurring with no injury or minor injury are the hallmark of OI, other non-mineralized tissues can be affected as well, and the pathological changes can be present in skin, tendons, eyes, teeth and blood vessels. Clinical manifestations are very heterogeneous, and numerous signs and symptoms such as blue sclera, deafness, abnormal teeth development, joint hypermobility, increased risk of hernias, capillary fragility, aneurysms, etc., have been observed. Sillence et al. classified four types of OI based on clinical and genetic findings in patients with OI [2]. Recently, a new classification was published, including 16 different genes responsible for this disease [3]. This diversity calls for a personalized treatment approach. Additionally, if OI patients are treated with early and lasting bisphosphonate therapy and nonelongating fixation implants, they may develop unique long-bone deformities and external/internal bone morphology, therefore, the surgical approach requires a personalized approach as well.

Osteogenesis Imperfecta Type III

Type III OI occurs in approximately 20% of all patients with OI [4]. All infants born with fractures and deformities who survive the perinatal period belong to this group. Most cases are presumably dominant new mutations in both type I collagen genes, however, rare autosomal recessive forms are also possible [5,6]. OI type III is usually recognized at birth because intrauterine fractures produce deformities of the long bones and severe skeletal changes. Clinical manifestations are very heterogeneous and numerous signs and symptoms such as blue sclera, deafness, abnormal teeth development, joint hypermobility, increased risk of hernias, capillary fragility, aneurysms, etc. Most patients have intrauterine

growth retardation, and further progressive growth failure continues during childhood as a result of long bone deformations and spinal involvement. A significant proportion of patients have large and asymmetric heads, while the face is usually triangular. In infancy, sclerae are often pale blue or grey but they regain normal color by puberty. The maxilla is frequently posteriorly inclined, and most craniofacial size measurements are reduced [7]. During the first 10 years of life, the number of fractures and the extent of skeletal changes is approximately the same in type III and type IV of OI. About 30% of patients experience recurrent abdominal pain due to chronic constipation and pelvic deformity with severe acetabular protrusion [8]. Occasionally, congenital cardiac malformations, hemihypertrophy, papillary calcifications or kidney stones, as well as hypercalciuria, are seen [9,10]. The development of all motor milestones is significantly delayed compared with the type I and IV, with a discrepancy between static and dynamic milestones [11]. A child who can sit by the age of nine to ten months is likely to achieve independent walking [12,13]. According to a study by Engelbert et al, 27% of children with OI type III achieved household ambulation with crutches, whereas 45% were restricted to the use of wheelchairs [12]. Most children who are independent in ambulation have poor joint alignment, poor balance, and low endurance. Bending and angulations of the long bones, hip contractures, and pelvic deformities are present in the most severe cases, hindering independent walking. Joint laxity results in hyperextension and valgus position of the knees and feet. Muscle strength is usually severely decreased, with a muscular imbalance around the hip joint [14]. Children who are not ambulatory usually have joint contractures and malalignment of the upper extremities, leading to recurrent fractures [15]. Osteopenia and joint hyperlaxity often lead to progressive kyphoscoliosis and chest wall abnormalities.

At birth, undermineralized calvarium, and elongated long bones and ribs are seen radiologically. Angulation deformities resulting from poor healing often lead to further pathological fractures. Recurrent microfractures of the growth plate appear by the age of two years. They form cystic structures in the epiphyseal region of the long bones (popcorn epiphyses) and arrest growth [16]. Radiographic findings of six or more biconcave vertebrae before puberty is a sign that severe scoliosis is likely to develop [17]. Multiple microfractures of the vertebral bodies lead to further deformities by damaging the vertebral growth plates. Low back pain resulting from vertebral compression fractures following minimal trauma is often experienced. Other complications, such as spondylolysis and spondylolisthesis are also seen. The most severe neurological complications result from the craniocervical instability caused by laxity at the C1-C2 vertebrae. This can lead to the progressive shifting of the tip of the dens of the C2 vertebrae into the foramen magnum resulting in basilar invagination and compression of the medulla oblongata and the cervical part of the spinal medulla. Basilar invagination generally progresses slowly causing many neurological signs and symptoms, which can be very subtle at first [18-20]. The growth rate is severely reduced from birth to about 6-7 years of age and then stops completely [21]. Although severely short stature is almost always seen in type III OI, serum insulin-like growth factor (IGF) I is normal [21,22]. The extent to which the lifestyle of the patient is affected correlates with the severity of the physical impairments. Concerns about physical appearance are intensified in puberty, and depression related to feelings of inadequacy may appear in adulthood [23]. Early mortality in OI type III is due to respiratory illness or complications resulting from basilar invagination or injury. Intracranial hemorrhage can result even from minor trauma and can cause rapid death [19,24].

Case report - personalized surgery approach

To illustrate the importance of a personalized approach to OI treatment, we present the case of an 11-year-old girl, who had multiple surgeries with nonelongating implants during her childhood. At admission, she had a severe angular deformity of both femurs and the lower legs. When she presented to St. Catherine Specialty Hospital, she was unable to walk even with assistance and was confined to a wheelchair. Before admission to our hospital, she was treated with 18 cycles of pamidronate intravenous infusion. Blood sampling was performed before the surgery, and molecular genetic analysis confirmed the clinical diagnosis of OI (type III). The patient underwent two surgeries in our hospital, with a 5-month interval between them. During the first hospitalization, she underwent surgery on both femurs. Firstly, the titanium elastic stable intramedullary nail – ESIN (Nancy) was extracted from the left femur, then a double osteotomy was made of both femurs with fixation by Fassier–Duval telescopic intramedullary nails. Five months after the first hospitalization, the patient was once again admitted to St. Catherine Specialty Hospital for both lower legs surgery. Extraction of two elastic stable intramedullary nailings (Nancy) from the right tibia, double corrective osteotomy of the right and single corrective osteotomy of the left tibia, and fixation of both tibias with intramedullary Fassier–Duval telescopic nails was performed. Figures 1 and 2 show the patient's clinical status preoperatively and ~ 2 months after the second surgery. Radiographs confirmed a good mechanical axis and the correct position of distal threading. Ten weeks after the second surgery, she started to walk with a

Figure 1. Preoperative and postoperative radiograph of the right femur, anteroposterior view (a, b); preoperative and postoperative radiograph of the right femur, lateral view (c, d); preoperative and postoperative radiograph of the left femur, anteroposterior view (e, f); preoperative and postoperative radiograph of the left femur, lateral view (g, h).

Figure 2. Preoperative and postoperative radiograph of the right tibia, anteroposterior view. (a, b) Preoperative and postoperative radiograph of the right tibia, lateral view (c, d); preoperative and postoperative radiograph of the left tibia, anteroposterior view (e, f); preoperative and postoperative radiograph of the left tibia, lateral view (g, h).

walker on level surfaces, and 5 months after the second surgery, she was able to walk with crutches. Finally, 7 months after surgery, she walked independently or sometimes with crutches, and she is able to climb the stairs. A 2-year follow-up revealed no changes in the patient's status.

Discussion

Due to a plethora of clinical and genetic classifications with many types and subtypes of OI, it is not possible to make a single algorithm of treatment and a universal approach, which could be useful and successful for every patient. It is very important to take into consideration every patient individually and to make a particular plan of treatment for every single patient. The cornerstone of OI treatment in St Catherine Specialty Hospital is a personalized approach to every patient. Molecular analysis of our OI patient was performed before the surgery, and it confirmed the clinical diagnosis – OI type III. The molecular genetic analysis helps to understand general aspects of the disease and has a strong predictive value on the final therapeutic outcome. It has been suggested that the mutations closer to the carboxy-terminal end of the triple-helical domain, including glycine substitution, lead to more clinically severe OI than those towards the N-terminus [25]. Nevertheless, mutations positioned at the C-terminal end of the alpha2 chain are not only related to limb anomalies but also to intracranial hemorrhage [26]. During the surgery, there are three key points and goals: correction of the angular deformity, preparation of intramedullary canal and preparation of soft tissue. The patient who was presented is an example of a severe OI case. Angular deformities of her thighs and lower legs were very severe and, before admission to our hospital, she was treated with 18 cycles of intravenous infusions of pamidronate. Because of repeated treatment with antiresorptive therapy, the remodelation of her bones was slowed down, and the intramedullary canal was filled with sclerotic bone. The sclerotic intramedullary canal makes it very difficult to pass the narrow or obliterated sites of the diaphyseal bone. This can extend the duration of surgery and also lead to other complications including perforation of the bone cortex, malposition of the distal threading and increased blood loss. Preoperative radiographs presented that every segment was deformed in two different directions: procurvatum and varus. As a result, we obtained a new unique deformity of each bone, which required careful preoperative planning of the osteotomy level and the size/angle of the excised bone wedge. All of the presented deformities may look similar but it is impossible to find two exactly the same. Every segment required a personalized approach: preoperative planning, patient positioning on the operating table and, last but not the least, correction of the deformity. The case we presented represents an illustrative example of our approach to the treatment of severe forms of OI.

However, we expect that the efficiency of our treatment concept will be confirmed during a longer follow-up study of all OI patients treated in St. Catherine Specialty Hospital.

Note:

References

[1] Steiner RD, Pepin MG, Byers PH. Osteogenesis imperfecta. In: Pagon RA, Bird TD, Dolan CR, Stephens K, editors. GeneReviews. Seattle: University of Washington; 1993–2017.

[2] Sillence DO, Senn A, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. J Med Genet. 1979;16:101–16.

[3] Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet A. 2014;164:1470–81.

[4] Rowe WD, Shapiro RY. Osteogenesis imperfecta. In: Avioli L, Krane S, editors. Metabolic bone disease and clinical related disorders. 2nd ed. Philadelphia: WB Saunders; 1990. p. 659-701.

[5] Byers PH. Osteogenesis imperfecta. In: Royce PM, Steinmann B, editors. Connective tissue and its heritable disorders: molecular, genetic and medical aspects. New York (NY): Wiley-Liss; 1993. p. 317-50.

[6] Lund AM, Astrom E, Soderhall S, Schwartz M, Skovby F. Osteogenesis imperfecta: mosaicism and refinement of the genotype-phenotype map in OI type III. Mutations in brief. Hum Mutat. 1999;13:503.

[7] Jensen BL, Lund AM. Osteogenesis imperfecta: clinical, cephalometric, and biochemical investigations of OI types I, III, and IV. J Craniofac Genet Dev Biol. 1997;17:121-32.

[8] Lee JH, Gamble JG, Moore RE, Rinsky LA. Gastrointestinal problems in patients who have type III osteogenesis imperfecta. J Bone Joint Surg. 1995;77:1352-6.

[9] Vetter U, Pontz B, Zauner E, Brenner RE, Spranger J. Osteogenesis imperfecta: a clinical study of the first ten years of life. Calcif Tissue Int. 1992;50:36-41.

[10] Chines A, Bonicace A, McAlister W, Whyte M. Hypercalciuria in osteogenesis imperfecta: a follow-up stud to assess renal effects. Bone. 1995;16:333-9.

[11] Engelbert RH, Pruijs HE, Beemer FA, Helders PJ. Osteogenesis imperfecta in childhood: treatment strategies. Arch Phys Med Rehabil. 1998;79:1590-4.

[12] Engelbert RH, Uiterwaal CS, Gulmans VA, Pruijs H, Helders PJ. Osteogenesis imperfecta in childhood: prognosis for walking. J Pediatr. 2000;137:397-402.

[13] Daly K, Wisbeach A, Sanpera I Jr, Fixsen JA. The prognosis for walking in osteogenesis imperfecta. J Bone Joint Surg Br. 1996;78:477-80.

[14] Engelbert RH, van der Graaf Y, van Empelen R, Beemer FA, Helders PJ. Osteogenesis imperfecta in childhood: impairment and disability. Pediatrics. 1997;99:E3.

[15] Binder H, Conway A, Gerber LH. Rehabilitation approaches to children with osteogenesis imperfecta: a ten-year experience. Arch Phys Med Rehabil. 1993;74:386-90.

[16] Marini J, Gerber NL. Osteogenesis imperfecta: rehabilitation and prospects for gene therapy. JAMA. 1997;277:746-50.

[17] Ishikawa S, Kumar SJ, Takahashi HE, Homma M. Vertebral body shape as a predictor of spinal deformity in osteogenesis imperfecta. J Bone Joint Surg Am. 1996; 78:212-9.

[18] Engelbert RH, Gerver WJ, Breslau-Siderius LJ, van der Graaf Y, Pruijs HE, van Doorne JM, et al. Spinal complications in osteogenesis imperfecta: 47 patients 1-16 years of age. Acta Orthop Scand. 1998;69:283-6.

[19] Sillence DO. Craniocervical abnormalities in osteogenesis imperfecta: genetic and molecular correlation. Pediatr Radiol. 1994;24:427-30.

[20] Charnas LR, Marini JC. Communicating hydrocephalus, basilar invagination and other neurological features in osteogenesis imperfecta. Neurology. 1993;43:2603-8.

[21] Vetter U, Pontz B, Zauner E, Brenner RE, Spranger J. Osteogenesis imperfecta: a clinical study of the first ten years of life. Calcif Tissue Int. 1992;50:36-41.

[22] Lund AM, Muller J, Skovby F. Anthropometry of patients with osteogenesis imperfecta. Arch Dis Child. 1999;80:524-8.

[23] Cole DE. Psychosocial aspects of osteogenesis imperfecta: an update. Am J Med Genet. 1993;45:207-11.

[24] McAllion SJ, Paterson CR. Causes of death in osteogenesis imperfecta: J Clin Pathol. 1996;49:627-30.

[25] Bateman JF, Moeller I, Hannagan M, Chan D, Cole WG. Characterization of three osteogenesis imperfecta collagen alpha 1(I) glycine to serine mutations demonstrating a position-dependent gradient of phenotypic severity. Biochem J. 1992;288:131–5.

[26] Faqeih E, Roughley P, Glorieux F, Rauch F. Osteogenesis imperfecta type III with intracranial hemorrhage and brachydactyly associated with mutations in exon 49 of COL1A2. Am J Med Genet A. 2009;149:461–65.

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Pharmacogenomics – the key to personalized medicine.

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Abstract

Different rates of drug metabolism and the effect of commonly prescribed drugs are often seen in clinical practice. Some of these differences can be predicted if the patient's genetic profile is known by pharmacogenomic analysis, which done once, provides lifetime benefits. In the United States, adverse drug reactions are the fourth leading cause of death, costing their healthcare system about $136 billion annually. By implementing pharmacogenomic testing early in clinical algorithms, debilitating and potentially life-threatening side-effects can be predicted and avoided, which is particularly important in settings of pain therapy and anesthesia. In St. Catherine Specialty Hospital, this approach is readily advocated for our patients. Through the use of the RightMed panel, 25 genes coding for enzymes and other proteins important for drug function, are analyzed, and a pharmacogenomic-driven approach is taken by selecting the right drug, in the right dose, for the right patient.

Keywords: pharmacogenomics, individualized medicine, personalized medicine, analgesia

Introduction

Personalized medicine, considering the genetic profile of the patient, lifestyle, and environmental factors, has the potential to add new value to patient care and to change the current one-size-fits-all approach by utilizing pharmacogenomic data. Pharmacogenomics focuses on identifying genetic variants that influence the metabolism and effects of drugs, their pharmacokinetics and pharmacodynamics, with the ultimate goal of understanding individual patient's drug and dose requirements based on their own genetic profile. The terms pharmacogenetics and pharmacogenomics appear similar at first, however, pharmacogenetics explains a single gene-drug interaction, whereas pharmacogenomics is a broader field in which multiple genes are considered [1]. Genetic polymorphisms were first associated with different rates of substrate drug metabolism back in the 1970s in CYP2D6 research [2]. Nowadays, custom single nucleotide polymorphism assay chips are commercially available for anyone who wants to learn their pharmacogenetic profile, and for those clinicians who want to use this approach to tailor their patient's therapy. The latest data from the United States indicates that adverse drug reactions (ADRs) account for up to 7% of all hospital admissions, up to 20% of re-admissions and are the fourth leading cause of death. Their total estimated cost is $136 billion annually [3-6]. Genetic factors can account for up to 95% of an individual's drug response and are estimated to contribute to as much as 20% of the total number of reported ADRs [3,4,7]. These numbers call for a reconsideration of current healthcare strategies and the development of new treatment tools that would reduce the risk for patient's health and the additional treatment costs that predictable ADRs generate.

Background

It is well known that carriers involved in drug transport through different physiological barriers and enzymes that metabolize drugs are proteins, as well as most drug target receptors. This core principle is used in pharmacogenomics, as the variety of observed end-protein activity is influenced by underlying genetic polymorphisms. Therefore, there cannot be a one-size-fits-all drug for a single condition [8]. In pharmacogenomic studies, different alleles are analyzed to find single nucleotide polymorphisms (SNPs) [9]. SNPs make the difference in observed drug effect by modeling the activity of their protein product (metabolizing enzyme, transporter protein, drug-receptor, or other proteins not directly related to the drug). The end protein product can therefore be of normal, increased or decreased function/phenotype, based on various allele combinations. The results of these molecular analyses can be challenging

for a clinician to understand, as the integration of genetics in clinical medicine is a new, interdisciplinary field. Therefore, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has established a standardized method for molecular pharmacogenomic analysis report terminology in order to ease the introduction of report interpretation for clinicians and patients. The standardized reporting should be oriented toward indicating the end-protein function, depending on the detected genotype [10].

For a proper introduction of pharmacogenomic guided therapeutic interventions, comprehensive guidelines are needed. CPIC has published 25 clinical guidelines designed to help clinicians optimize drug therapy according to pharmacogenomic analysis [11] (Table 1).

Table 1. Genes-drugs interaction with the highest level of clinical relevance (Clinical Pharmacogenetics Implementation Consortium guidelines recommend a change in prescribing of the affected drug based on evidence that include consistent results from well-designed, well-conducted studies).

Classification of genes	Example of genes	Drug examples	Effect
I. Genes involved in the first phase of drug metabolism – cytochrome P450 enzymes	CYP2C9	Phenytoin, warfarin	Certain genotype may influence drug substrate metabolism – consequent changes in drug efficacy and safety
	CYP2C19	Amytriptiline (es)citalopram, sertraline, clopidogrel, voriconazole	
	CYP2D6	Tricyclic antidepressants, atomoxetine, codeine, tramadol, fluvoxamine, paroxetine, ondansetron, tropisetron, tamoxifen	
	CYP3A5	Tacrolimus	
	CYP4F2	Warfarin	
II. Genes involved in the second phase of drug metabolism	UGT1A1	Atazanavir	Safety – risk of hyperbilirubinemia
III. Other genes involved in drug metabolism	TPMT/NUDT15	Azathioprine[†], mercaptopurine[†], thioguanine[†]	Efficacy and safety
	DPYD	Capecitabine, tegafur, fluorouracil	Safety – increased drug toxicity
IV. Genes for drug transporters	SLCO1B1	Simvastatin	Safety – risk of myopathy
V. Genes for drug targets	VKORC1	Warfarin	Efficacy
	CFTR	Ivacaftor[‡]	Efficacy
VI. Genes for other proteins important for drug action and safety	HLA-A	(Ox)carbamazepine	Safety – increased risk of SJS/TEN
	HLA-B	(Ox)carbamazepine[‡], abacavir[‡], fosfo(phenytoin), allopurinol	Safety – increased risk of SJS/TEN
	IFNL3	Peginterferon-alfa2a and 2b, ribavirin	Efficacy
	G6PD	Rasburicase[‡]	Toxicity – risk of hemolysis
	CACNA1S/RyR1	Inhalational anesthetics/suxamethonium	Toxicity – increased risk of malignant hyperthermia

†Testing recommended (according to FDA).
‡Testing required (according to FDA).

Therefore, there is a growing need for high-quality studies that would reinforce current concepts and possibly provide new insight into the combined effect of multiple drug-metabolizing enzymes and receptors on a given drug.

Pain therapy

In daily clinical practice, pain is one of the most commonly treated symptoms. It can be provoked by a plethora of underlying medical conditions that require different treatment strategies. However, successful pain management requires the delivery of analgesia with minimal risk of ADRs. It is often observed that patient's responses to pain medications vary, with almost 50% of patients experiencing inadequate pain relief and serious ADRs with commonly used perioperative analgesics [12]. The direct and indirect cost of chronic pain management ranges from $560 to 635 billion annually in the United States [13-15]. Some of the most commonly used analgesics have available pharmacogenomic information in their FDA label or in CPIC/ Dutch Pharmacogenetics Working Group (DPWG) clinical guidelines, including opioids (codeine, oxycodone, tramadol), anti-inflammatory (celecoxib) and neuropathic pain drugs (tricyclic antidepressants). It should be stated that knowing the genetic profile alone is not enough to completely alleviate pain in patients suffering from various painful conditions. Other factors such as environmental, age, sex, previous medical conditions and lifestyle greatly contribute to the individual sensation of pain [16]. Therefore, a combined approach that includes both the clinical assessment on a patient-to-patient basis and genetic testing is needed to precisely select the optimal strategy for analgesic therapy. Pain therapy is also one of the cornerstones of safe anesthesia. General anesthesia commonly combines various drugs such as volatile anesthetics, hypnotic-sedative agents, muscle relaxants, and opioid analgesics. Although efficient and safe anesthesia is grounded on clinical protocols, which take into consideration relevant procedure-, patient- and drugs-related factors, direct drug levels monitoring and automatic closed-loop control; various polymorphisms of genes encoding for anesthetic drug molecular targets, as well as their transporters and metabolic enzymes, might change a drug pharmacodynamic or pharmacokinetic characteristics, thus influencing the clinical features of anesthesia [17]. Some of the most dangerous complications, like malignant hyperthermia, have a clear genetic background for which a clinical guideline has been established by CPIC [18].

Opioids

Moderate quality evidence has shown that the variability of the analgesic effect is affected by genetic variants in opioid receptors M1 (OPRM1) and catechol-O-methyltransferase (COMT). Polymorphisms of the gene encoding for COMT, an enzyme that degrades catecholamines, are most frequently linked to divergent pain processing [19]. The most studied functional SNP of COMT is rs4680, where A to G transition results in a Val to Met amino-acid substitution, and lower enzymatic activity, as demonstrated in several studies where AA genotype carriers had increased sensitivity to the analgesic effects of opioids compared to AG and GG genotypes [20-22]. Over 100 allele variants in the OPRM1 gene encoding for the M opioid receptor (MOR) have been identified. SNP rs1799971 (118A>G) was related to reduced analgesic effect and requirements for higher doses of morphine [23]. The frequency of the OPRM1 188A>G variant differs between ethnic populations: 35–50% in individuals of Asian descent, 15.4% in individuals of European descent, 14% in individuals of Hispanic descent and 4.7% in those of African descent [24]. A member of the cytochrome P450 family of metabolizing liver enzymes, CYP2D6, is responsible for metabolizing a number of opioid analgesics, including codeine, oxycodone and tramadol. In regard to detected allelic variants, its function/ phenotype can be categorized as poor, intermediate, rapid or ultrarapid [25]. The analgesic effects of codeine and tramadol which are metabolized by CYP2D6, are attributed to their O-demethylation to a more potent MOR agonist, therefore they are "activated" when they undergo CYP2D6 metabolization. Thus, the effect of different phenotypes is reciprocal, where poor metabolizers are at an increased risk of inadequate therapeutic response and ultrarapid metabolizers have a higher risk of adverse effects and overdose. This observation is influenced by changes in their pharmacokinetics, altering the concentration of their active metabolites. A pharmGKB guideline was developed to aid in clinical interpretation and decision-making of CYP2D6 phenotype for tramadol and codeine. For ultrarapid metabolizers, alternative analgesics are suggested [26]. Previous reports warned of respiratory depression and death in children taking codeine who were identified as CYP2D6 ultrarapid metabolizers [27]. Because of the risks associated with CYP2D6 ultrarapid metabolism, breastfeeding is not recommended during treatment with codeine or tramadol. Opioids are commonly used in clinical practice and they have a well-known side-effect profile that can, however, be predicted and reduced if the pharmacogenomic guided approach is used.

Non-steroid anti-inflammatory drugs (NSAIDs)

NSAIDs include two big groups of drugs: non-selective cyclooxygenase inhibitors and selective cyclooxygenase-2 inhibitors, such as etoricoxib and

celecoxib. Both of these groups have analgesic and anti-inflammatory effects. The side effect profile of NSAIDs includes gastrointestinal and cardiovascular ADRs, therefore it is important to use them with care as they are readily available over-the-counter. It is particularly important to consider their side-effect profile in older populations since they are at an already increased risk for gastrointestinal and cardiovascular disease [28-31]. Different bioavailability based on CYP2C8 genotype was shown to have a role in patients developing potentially serious adverse drug reactions with prolonged use of NSAIDs [32]. SNPs of another member of the cytochrome P450 enzyme family, CYP2C9, have been found to influence the metabolism rate of celecoxib and flurbiprofen. For patients who have a determined poor metabolizer phenotype (CYP2C9 *3/*3) a 50% reduction in starting dose has been suggested to avoid potential side effects, however, it is not a part of any official guideline [33]. Another study found an increase in gastrointestinal tract bleeding risk in patients carrying CYP2C8*3 and CYP2C9*2 alleles when using NSAIDs that are the substrate of both of those enzymes, such as ibuprofen and diclofenac [34]. This evidence still needs to be reinforced by more robust clinical studies. But, considering the potential detrimental effect that NSAID ADRs can have on the elderly population the changes in their metabolism induced by these SNPs should be considered if available.

Malignant hyperthermia

In anesthesiology, the most important pharmacogenetic influence on the safety of volatile anesthetics and succinylcholine is the prediction of malignant hyperthermia risk. Influenced by SNPs in RYR1 and CACNA1S genes, which are inherited in an autosomal-dominant pattern, a heterozygous genotype is considered to be diagnostic of the trait [18]. The variants in RYR1 and CACNA1S genes under consideration here predispose individuals to a severe and sometimes fatal hypermetabolic reaction with symptoms that include muscle rigidity, high fever, and a fast heart rate, and can include rhabdomyolysis and high blood potassium. For individuals with SNPs in the stated genes, succinylcholine and volatile anesthetics should be avoided due to increased malignant hyperthermia susceptibility. The presence of a single pathogenic variant in RYR1 or CACNA1S found by molecular genetic testing is considered diagnostic for the trait [35].
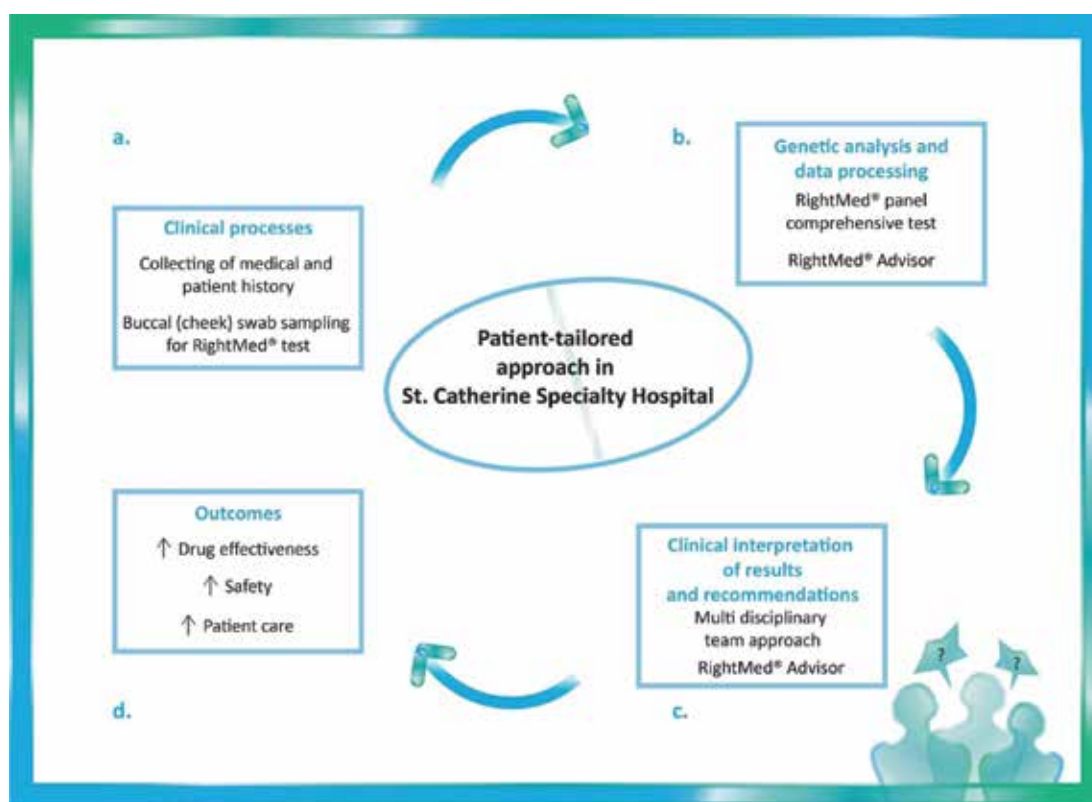

Figure 1. A schematic representation of implementation personalized pharmacogenomic-based treatment and healthcare system. The first step is to collect medical and patient history via a specific form followed by a buccal swab for DNA sampling for RT-PCR. b. RightMed® panel comprehensive test processes the PGx results using an algorithm. RightMed® Advisor is being generated here. c. Results are then interpreted by a multi-disciplinary team. In addition to drug-gene interactions, the RightMed® Advisor platform checks for drug-drug, drug-food (supplement) interactions. d. The results aid in providing better patient care, superior therapy outcome and greater drug effectiveness while at the same time reducing the rate of ADRs.

Our approach

At St. Catherine Specialty Hospital, where the personalized approach to patient care is pioneered, patients referred for pharmacogenomic testing are first interviewed by one of our specialists in the outpatient clinic (Figure 1). A detailed patient history focusing on current and previous drug therapy is taken, after which a buccal swab is performed in order to obtain a sample of the patient's DNA. The obtained DNA is then analyzed using the RightMed panel developed by OneOme in collaboration with Mayo Clinic. The panel determines SNPs using a TaqMan real-time PCR method and CNV analysis. The 25 analyzed genes include genes responsible for the synthesis of enzymes included in the first phase of drug metabolism (cytochrome P450 family), enzymes included in the second phase of drug metabolism (TPMT, UGT1A1), other enzymes included in drug metabolism (DPYD, VKORC1, NUDT15), drug transporters (SLC6A4, SLCO1B1), drug receptors (HTR2A, HTR2C, DRD2, OPRM1, GRIK4, COMT), other proteins important for drug function (IFNL4, HLA-A, HLA-B). The results are then generated as individual test reports that are interpreted by a genetic counseling team in St. Catherine Specialty Hospital. Once the report is made, the patient is scheduled for a follow-up with one of our specialists to determine if any corrections have to be made to their current therapy and to provide suggestions for potential future therapy needs based on the clinical assessment.

Conclusions

Pharmacogenomic testing performed once can provide lifetime benefits. Although more effort is still needed to implement pharmacogenomics in daily clinical practice, evidence suggests that personalized medicine is the treatment approach of the future and pharmacogenomic testing is at the center of it, with the benefits of cost reductions and improved quality of care which every healthcare system should value. In the future, digital tools should be made available for all healthcare professionals that come across comprehensive pharmacogenomic test results to ease their interpretation and reinforce drug prescription on the basis of individualized medicine.

Note:

Pharmacogenomics – the key to personalized medicine is an excerpt from the texts prepared for: Personalized medicine, scientific journal (Bach-Rojecky L, Vađunec D, Žunić K, Kurija J, Šipicki S, Gregg R, Mikula I, Primorac D. Continuing war on pain: a personalized approach to the therapy with nonsteroidal anti-inflammatory drugs and opioids. Per Med. 2019;16(2):171-184. doi: 10.2217/pme-2018-0116.) and Pharmacogenomics, scientific journal (Primorac D, Bach-Rojecky L, Vađunec D, Juginović A, Žunić K, Matišić V, Skelin A, Arsov B, Boban L, Erceg D, Erceg Ivkošić I, Molnar V, Ćatić J, Mikula I, Boban Lj, Primorac L, Esquivel B, Donaldson M. Pharmacogenomics at the center of precision medicine: challenges and perspective in an era of Big Data. Pharmacogenomics. 2020 Jan;21(2):141-156. doi: 10.2217/pgs-2019-0134.) in which the genetic basis of pain and pain therapy and a comprehensive review of implementation of pharmacogenomics to clinical practice were prepared and presented in full.

References:

[1] Kaye, A.D., Mahakian, T., Kaye, A.J., Pham, A.A., Hart, B.M., Gennuso, S., Cornett, E.M., et al. Pharmacogenomics, precision medicine, and implications on anesthesia care. Best Pract Res Clin Anaesthesiol. 2018;32(2):61–8.

[2] Ingelman-Sundberg, M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J. 2005;5:6–13.

[3] Lazarou, J., Pomeranz, B.H., Corey, P.N. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. JAMA. 1998;279(15):1200–1205.

[4] Plumpton, C.O., Roberts, D., Pirmohamed, M., Hughes, D.A. A systematic review of economic evaluations of pharmacogenetic testing for prevention of adverse drug reaction. Pharmacoeconomics. 2016;34(8):771–793.

[5] Spear, B.B., Heath-Chiozzi, M., Huff, J. Clinical application of pharmacogenetics. Trends Mol. Med. 2001;7(5):201–204.

[6] Adams, J. Pharmacogenomics and personalized medicine. Nat Educ. 2008;1(11):194.

[7] Kalow, W., Tang, B.K., Endrenyi, L. Hypothesis: comparisons of inter- and intra-individual variations can substitute for twin studies in drug research. Pharmacogenetics. 1998;8(4):283–289.

[8] Höppner, W., Primorac, D. Pharmacogenetics in clinical practice experience with 16 commonly used drugs. St. Catherine Hospital, Zagreb, Berlin, Hamburg, 2016.

[9] Dib, P., Tung, S.R., Dib, S.A., Hamdy, A., Kitzmiller, J.P.. Pharmacogenomics applications in perioperative medicine. J Transl Sci. 2018;4(5):1–6.

[10] Caudle, K.E., Dunnenberger, H.M., Freimuth, R.R., Peterson, J.F., Burlison, J.D., Carrillo, M.W., Scott, S.A., et al. Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC). Genet Med. 2017;19(2):215–23.

[11] Clinical Pharmacogenetics Implementation Consortium 2019 (2019). https://cpicpgx.org/. Accessed 16 February 2021

[12] Trescot, A.M., Faynboym, S. A review of the role of genetic testing in pain medicine. Pain Physician. 2/14;17(5):425–445.

[13] Bach-Rojecky, L., Vadunec, D., Zunic K., et al. Continuing war on pain: a personalized approach to the therapy with nonsteroidal anti-inflammatory drugs and opioids. Per Med. 2019;16(2):171–184.

[14] Primorac, D., Bach-Rojecky, L. Could a personalized approach to therapy end the war on pain? Pract Pain Manag. 2019;19(3):50–53.

[15] Pizzo, P.A., Clark, N.M. Alleviating suffering 101 – pain relief in the United States. N Engl J Med. 2012;366(3), 197–199.

[16] Dean, E., Söderlund, A. What Is the Role of Lifestyle Behaviour Change Associated with Non-Communicable Disease Risk in Managing Musculoskeletal Health Conditions with Special Reference to Chronic Pain? BMC Musculoskelet Disord. 2015;16:87.

[17] Bach-Rojecky, L., Čutura, T., Lozić, M., Husedžinović Kliškinjić, I., Matišić, V., Primorac, D. Personalized Anesthetic Pharmacology. In: Personalized Medicine in Anesthesia, Pain and Perioperative Medicine (Editor: Ali Dabbagh). Springer Nature Switzerland, Switzerland, 2021.

[18] Gonsalves, S.G., Dirksen, R.T., Sangkuhl, K., Pulk, R., Alvarellos, M., Vo, T., Hikino, K., et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for the use of potent volatile anesthetic agents and succinylcholine in the context of RYR1 or CACNA1S genotypes. Clin Pharmacol Ther. 2019;105(6):1338–44.

[19] Diatchenko, L., Fillingim, R.B., Smith, S.B., Maixner, W. The phenotypic and genetic signatures of common musculoskeletal pain conditions. Nat Rev Rheumatol. 2013;9(6):340–350.

[20] Ahlers, S.J., Elens, L.L., van Gulik, L., van Schaik, R.H., van Dongen, E.P.A., Bruins, P., Tibboel, D., et al. The Val158Met polymorphism of the COMT gene is associated with increased pain sensitivity in morphine-treated patients undergoing a painful procedure after cardiac surgery. Br J Clin Pharmacol. 2013;75(6):1506–1515.

[21] Reyes-Gibby, C.C., Shete, S., Rakvag, T., Bhat, S.V., Skorpen, F., Bruera, E., Kaasa, S., et al. Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene. Pain. 2007;130(1–2):25–30.

[22] Candiotti, K.A., Yang, Z., Buric, D., Arheart, K., Zhang, Y., Rodriguez, Y., Gitlin, M.C., et al. Catechol-o-methyltransferase polymorphisms predict opioid consumption in postoperative pain. Anesth. Analg. 2014;119(5):1194–1200.

[23] Owusu Obeng, A., Hamadeh, I., Smith, M. Review of opioid pharmacogenetics and considerations for pain management. Pharmacotherapy. 2017;37(9):1105–1121.

[24] Bell, G.C., Donovan, K.A., McLeod, H.L. Clinical implications of opioid pharmacogenomics in patients with cancer. Cancer Control 2015;22(4):426–432.

[25] Ting, S., Schug, S. The pharmacogenomics of pain management: prospects for personalized medicine. J Pain Res.2016; 9:49–56 (2016).

[26] Pharm GKB. Tramadol overview. https://www.pharmgkb.org/chemical/PA451735/overview (2020). Accessed 16 February 2021.

[27] Koren, G., Cairns, J., Chitayat, D., Gaedigk, A., Leeder, S.J. Pharmacogenetics of morphine poisoning in a breastfed neonate of a codeine-prescribed mother. Lancet. 2006;368(9536):704.

[28] Sostres, C., Gargallo, C.J., Arroyo, M.T., Lanas, A. Adverse Effects of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs, Aspirin and Coxibs) on Upper Gastrointestinal Tract. Best Pract Res Clin Gastroenterol. 2010;24:121–132.

[29] Gunter, B.R., Butler, K.A., Wallace, R.L., Smith, S.M., Harirforoosh, S. Non-Steroidal Anti-Inflammatory Drug-Induced Cardiovascular Adverse Events: A Meta-Analysis. J Clin Pharm Ther. 2017;42:27–38.

[30] Grosser, T., Ricciotti, E., FitzGerald, G.A. The Cardiovascular Pharmacology of Nonsteroidal Anti-Inflammatory Drugs. Trends Pharmacol. Sci. 2017;38:733–748.

[31] Steinmeyer, J., Bock, F., Stöve, J., Jerosch, J., Flechtenmacher, J. Pharmacological Treatment of Knee Osteoarthritis: Special Considerations of the New German Guideline. Orthop Rev. 2018;10:7782.

[32] Davies, N.M., Skjodt, N.M. Clinical pharmacokinetics of meloxicam. A cyclo-oxygenase-2 preferential nonsteroidal anti-inflammatory drug. Clin Pharmacokinet. 1999;36(2):115–126.

[33] Olkkola, K.T., Brunetto, A.V., Mattila, M.J. Pharmacokinetics of oxicam nonsteroidal anti-inflammatory agents. Clin Pharmacokinet. 1994;26(2):107–120.

[34] Palma-Aguirre, J.A., Lopez-Gamboa, M., Carino, L., Burke-Fraga, V., Gonzalez-De La Parra, M. Relative bioavailability of two oral formulations of piroxicam 20 mg: a single-dose, randomized-sequence, open-label, two-period crossover comparison in healthy Mexican adult volunteers. Clin Ther. 2010;32(2): 357–364.

[35] Rosenberg, H., Sambuughin, N., Riazi, S., Dirksen, R. Malignant Hyperthermia Susceptibility. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020. 2003 [Updated 2020 Jan 16] Available from: https://www.ncbi.nlm.nih.gov/books/NBK1146/

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COVID-19 severity determinants – modulating effect of environmental factors.

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Abstract

The new coronavirus that appeared in 2019 (SARS-CoV-2) caused the COVID-19 pandemic, which is responsible for over a million confirmed deaths in 2020, making it the most important event of the 21st century thus far and making SARS-CoV-2 the most scientifically studied virus. Most of those infected with the SARS-COV-2 virus (about 80%) recover quickly from the disease, showing minimal signs of inflammation, mostly similar to the common cold. We analyzed the possible explanations of this observation and demonstrated the association of relative humidity, temperature and IgG N-glycosylation with COVID-19 severity.

Keywords: SARS-CoV-2, pandemic, humidity, temperature, glycans

Introduction

The coronavirus is one of the major pathogens responsible for respiratory infections. Seven known coronaviruses cause disease in humans. SARS-CoV and MERS-CoV viruses cause the so-called severe acute respiratory syndrome (SARS), while four other coronaviruses that cause human diseases (HCoV-OC43, HCoV-229E, HCoV-NL63 and HCoV-HKU1) lead to mild upper respiratory tract infections, against which most adults have antibodies [1, 2]. The new coronavirus that appeared in 2019 (SARS-CoV-2) caused the COVID-19 pandemic, responsible for over a million confirmed deaths in 2020, making it the most important event of the 21st century thus far and making SARS-CoV-2 the most scientifically studied virus. The genome of the SARS-CoV-2 virus is composed of a single-stranded (positive-sense) ribonucleic acid (RNA) molecule and contains 29,903 nucleotides [3]. Using a special spike-like glycoprotein on its surface, the SARS-CoV-2 virus binds itself to angiotensin-converting enzyme (ACE2) to enter human cells, mainly in the respiratory system, where it successfully uses the cell structures to produce a new generation of viruses [4]. The clinical presentation of patients infected with the SARS-COV-2 is diverse and the symptoms that may occur include: fever, fatigue, dry cough, muscle pain and dyspnea (difficulty breathing), while the less common symptoms include: diarrhea and nausea, headache, hemoptysis (coughing up blood), productive cough and chest pain [5-7]. However, most patients infected with SARS-COV-2 (about 80%) recover quickly from the disease and show minimal signs of inflammation, mostly similar to the common cold. Many factors have been proposed as clinical predictors of disease progression including obesity, diabetes, hypertension, kidney, previous cardiovascular disease and age [8, 9].

Mucosal barrier

Epidemiological data from several sources show that transmission of SARS-CoV-2 is more efficient in cold and dry climate than in warm and humid locations [10, 11]. The majority of respiratory viruses demonstrate seasonality in their epidemiologic peaks, which suggests an important role of the environment for viral transmission [12]. Possible explanations for this phenomenon include indoor crowding in the cold months, effects of temperature and humidity on stability of viral particles and inactivation of the mucosal barrier of the respiratory tract. If the mucosal barriers dry-out, they cannot perform their protective functions [13]. It is, therefore, necessary to stay well hydrated to maintain the structural integrity and enable the constant flow of mucins that carry viruses and other pathogens out of

the airways [14]. Animal studies of influenza mortality demonstrated that increasing relative humidity from 20% to 50% significantly decreased mortality [15]. Another study examining this topic found that indoor relative humidity of over 40% significantly reduces the infectivity of aerosolized influenza virus particles [16]. These findings demonstrate the important role of relative humidity on viral transmissibility, which can be attributed to either their inactivation or to the preserved integrity of the mucosal barrier.

IgG N-glycome

IgG is the key effector of acquired humoral immunity. Synthesized by B-lymphocytes, IgG binds to its specific antigen and clears it out of our system by activating other immune cells. IgG glycome composition is an essential component of the immune system that regulates inflammation at multiple levels. Its role as both a biomarker and a functional effector of inflammation that contributes to the development of different inflammatory diseases has been established in the literature [17-19]. Therefore, our international group of authors analyzed the total IgG N-glycome composition of three COVID-19 patient cohorts from Spain, Italy and Portugal. 167 patients with mild and 166 patients with severe COVID-19 were included. Disease severity was defined in regard to intensive care unit admission and the need for mechanical ventilation. The results demonstrated a significant difference in the IgG glycome composition in severe and mild COVID-19 patients. A consistent decrease in the level of bisecting N-acetylglucosamine (GlcNAc) in severe cases was observed in all cohorts (meta-analysis effect = - 0.34; adjusted meta-analysis p= 0.009). Galactosylation was also consistently decreased in severe cases in all three cohorts, but the statistical significance of this difference was observed only for monogalactosylation in the Barcelona cohort. Consistent changes in the levels of sialylated and fucosylated IgG glycan structures between mild and severe COVID-19 cases were not detected.

Despite the small size of each cohort, the decreased level of bisecting GlcNAc in severe patients was statistically significant even after adjusting for multiple testing (adjusted meta-analysis p = 0.009). Higher levels of bisecting GlcNAc on IgG are often associated with increased FcγRIII binding and enhanced antibody-dependent cell cytotoxicity (ADCC), explaining more pro-inflammatory effector functions of IgGs [20-21].

The cross-sectional design of this study did not allow us to distinguish whether the observed associations reflected a pre-existing risk factor or rapid changes in IgG glycosylation that occurred during the natural course of the disease. This question will be addressed in future studies. The observed differences are not large enough to suggest the use of IgG glycome as a predictor of COVID-19 severity, but it is intriguing to hypothesize that changes in the IgG glycome that lead to the loss of its immunosuppressive potential may be one of the molecular mechanisms behind environmental risk factors for severe COVID-19. We do, however, know that age and adiposity are the main environmental factors that drive the decrease in IgG glycosylation implicating that the observed changes may be individual and partially depending on these and other factors [22-23]. IgG glycome composition is strongly associated with age, so it is very hard to exclude confounding factors with some other age-related changes, but the fact that people with severe COVID-19 had "older" IgG glycome composition suggests the need for further research in this direction.

Seasonality of SARS-CoV-2

Aiming to evaluate the seasonal nature of COVID-19, we evaluated the disease course in 6,914 individuals from nine cohorts admitted to hospitals in Europe and China from the beginning of the pandemic until July 2020. To avoid sampling bias, all hospitalizations that resulted in either death or medical discharge were included in the analysis. Patients with confirmed diagnosis of COVID-19 by polymerase chain reaction testing of a nasopharyngeal sample and/or a clinically/radiologically diagnosis of COVID-19 at the time of admission were included. Patients were not followed after discharge, but COVID-19 related early readmissions were considered as part of the COVID-19 course.

A meta-analysis of the effect of admission date on the mortality was performed and demonstrated a weighted average decrease in mortality odds across all studied hospitals of 1.9% per day. The most significant change was observed in Barcelona, where mortality odds decreased by 4.1% per day (p < 0.001).

Our model included age as a co-variate, so this change is unlikely to be accounted for by a change in the age of patients. To further confirm that age was not underlying the observed changes, we analyzed the age of patients admitted to hospitals in different periods and demonstrated that change in the age of patients was not a factor that could explain the observed decrease in mortality. The decrease in lengths of hospitalization was also statistically significant. The odds to need intensive care decreased in all hospitals in Europe and were individually statistically significant in all hospitals besides Bergamo, Helsinki, and Zagreb. A meta-analysis of European hospitals estimated that the odds to need intensive care decreased by 2.2% per day of change in the admission date and the odds to need mechanical ventilation decreased 2.1% per day of change in the admission date. Additionally,

we correlated the observed changes with local ambient temperature. To evaluate whether the temperature change may have been responsible for the observed changes in disease severity, we modeled mortality with the ambient temperature instead of the admission date. The results suggest a strong effect of ambient temperature on the mortality risk (OR = 0.854 per 1°C; CI = 0.773–0.944; p = 0.007).

The main limitation of this study is sampling bias. By focusing on the individual progression of the disease in already hospitalized patients, we excluded effects of the unknown number of true infections on national mortality rates, and we still cannot exclude the possibility that some other unidentified external factors (including confinement and social distancing, improvement and compliance of prevention and environmental hygiene protocols, and even decreased air pollution, which could have progressively affected the severity of patients arriving to the hospital) were affecting the composition of hospitalized patient cohorts and contributing to the decreased COVID-19 severity and mortality. Nevertheless, the data suggest that, in addition to affecting viral transmission, environmental factors also play an important role in already infected patients.

Conclusion

The COVID-19 pandemic has upended all areas of life and stopped the way of life as we knew it. During the course of the past year, we embraced a much different lifestyle than what we were used to in order to reduce the spread of the virus and save as many lives as possible. The immense effort put into SARS-CoV-2 research resulted in the fastest vaccine development, emphasizing the value of science in our society. There are high hopes for a return to our lives as they were before the pandemic, but caution is still prudent until the majority of our population is vaccinated and/or recovered. With winter nearing its end and warm spring and summer weather approaching, we could expect a decrease in both patient count and symptom severity.

Note:

COVID-19 severity determinants – modulating effect of environmental factors is an excerpt from the texts prepared for: Journal of Global Health, scientific journal (Lauc G, Markotić A, Gornik I, Primorac D. Fighting COVID-19 with water. J Glob Health. 2020 Jun;10(1):010344. doi: 10.7189/jogh.10.010344.); Frontiers in medicine, scientific journal (Kifer D, Bugada D, Villar-Garcia J, Gudelj I, Menni C, Sudre C, Vučković F, Ugrina I, Lorini LF, Posso M, Bettinelli S, Ughi N, Maloberti A, Epis O, Giannattasio C, Rossetti C, Kalogjera L, Peršec J, Ollivere L, Ollivere BJ, Yan H, Cai T, Aithal GP,

Steves CJ, Kantele A, Kajova M, Vapalahti O, Sajantila A, Wojtowicz R, Wierzba W, Krol Z, Zaczynski A, Zycinska K, Postula M, Lukšić I, Čivljak R, Markotić A, Brachmann J, Markl A, Mahnkopf C, Murray B, Ourselin S, Valdes AM, Horcajada JP, Castells X, Pascual J, Allegri M, Primorac D, Spector TD, Barrios C, Lauc G.. Effects of Environmental Factors on Severity and Mortality of COVID-19. Front Med (Lausanne). 2021 Jan 20;7:607786. doi: 10.3389/fmed.2020.607786.); and Glycobiology, scientific journal (Petrović T, Alves I, Bugada D, Pascual J, Vučković F, Skelin A, Gaifem J, Villar-Garcia J, Vicente MM, Fernandes Â, Dias AM, Kurolt IC, Markotić A, Primorac D, Soares A, Malheiro L, Trbojević-Akmačić I, Abreu M, Sarmento E Castro R, Bettinelli S, Callegaro A, Arosio M, Sangiorgio L, Lorini LF, Castells X, Horcajada JP, Pinho SS, Allegri M, Barrios C, Lauc G.. Composition of the immunoglobulin G glycome associates with the severity of COVID-19. Glycobiology. 2020 Nov 10:cwaa102. doi: 10.1093/glycob/cwaa102.) in which the environmental factors and IgG glycome compositions in COVID-19 patients were studied and presented in full.

References

[1] He, F., Deng, Y., Li, W.. Coronavirus Disease 2019 (COVID-19): What we know? J Med Virol. 2020;14;jmv.25766. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.25766

[2] Gorse, G.J., Patel, G.B., Vitale, J.N., O'Connor, T.Z. Prevalence of Antibodies to Four Human Coronaviruses Is Lower in Nasal Secretions than in Serum. Clin Vaccine Immunol. 2010;17(12):1875–80.

[3] Wu, F., Zhao, S., Yu, B., Chen, Y.M., Wang, W., Song, Z.G., et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265–9.

[4] Gurwitz, D. Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. Drug Dev Res. 2020;ddr.21656. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ddr.21656

[5] Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China. JAMA. 2020;323(11):1061.

[6] Guan, W., Ni, Z., Hu, Y., Liang, W., Ou, C., He, J., et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;NEJMoa2002032.

[7] Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507–13.

[8] Sehra, S.T., Salciccioli, J.D., Wiebe, D.J., Fundin, S., Baker, J.F. Maximum Daily Temperature, Precipitation, Ultraviolet Light, and Rates of Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 in the United States, Clinical Infectious Diseases. 2020;71(9): 2482–2487
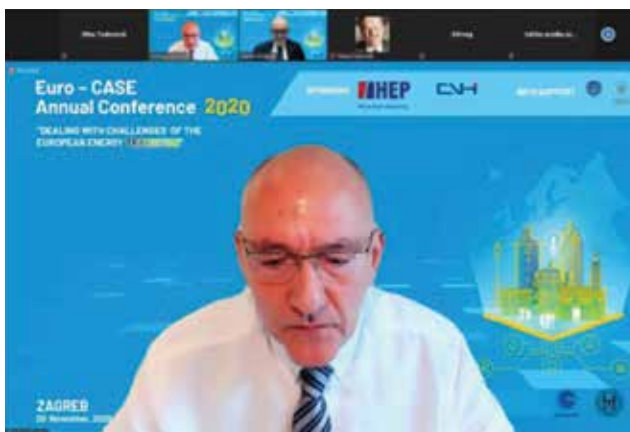
[9] Williamson, E., Walker, A. J., Bhaskaran, K. J., Bacon, S., Bates, C., Morton, C. E., Curtis, H. J., Mehrkar, A., et al. OpenSAFELY: factors associated with COVID-19-related hospital death in the linked electronic health records of 17 million adult NHS patients. MedRxiv, 2020.05.06.20092999. https://doi.org/10.1101/2020.05.06.20092999

[10] Wang, J., Tang, K., Feng, K., Lv, W. High temperature and high humidity reduce the transmission of COVID-19. SSRN Electron J. doi: 10.2139/ssrn.3551767

[11] Araujo, M.B., Naimi, B. Spread of SARS-CoV-2 Coronavirus likely to be constrained by climate. medRxiv 2020.03.12.20034728; doi: https://doi.org/10.1101/2020.03.12.20034728

[12] Moriyama, M., Hugentobler, W.J., Iwasaki, A..Seasonality of respiratory viral infections. Annu Rev Virol. 2020;7(1):83-101.

[13] Pflüger, R., Feist, W., Tietjen, A., Neher, A.Physiological impairments at low indoor air humidity. Gefahrst Reinhalt Luft. 2013;73:107-8.

[14] Wheeler, K.M., Cárcamo-Oyarce, G., Turner, B.S., Dellos-Nolan, S., Co, J.Y., Lehoux, S., et al. Mucin glycans attenuate the virulence of Pseudomonas aeruginosa in infection. Nat Microbiol. 2019;4:2146-54.

[15] Kudo, E., Song, E., Yockey, L.J., Rakib, T., Wong, P.W., Homer, R.J., et al. Low ambient humidity impairs barrier function and innate resistance against influenza infection. PNAS. 2019;116(22):10905-10910.

[16] Noti, J.D., Blachere, F.M., McMillen, C.M., Lindsley, W.G., Kashon, M.L., Slaughter, D.R., et al. High humidity leads to loss of infectious influenza virus from simulated coughs. PLoS One. 2013;8:e57485. 10.1371/journal.pone.0057485

[17] Lauc, G., Pezer, M., Rudan, I., Campbell, H. Mechanisms of disease: The human N-glycome. Biochimica et Biophysica Acta - General Subjects. 2016;1860(8):1574–1582.

[18] Nimmerjahn, F., Ravetch, J. V. Anti-inflammatory actions of intravenous immunoglobulin. Annu Rev Immunol. 2008;26:513–533.

[19] Seeling, M., Brückner, C., Nimmerjahn, F. Differential antibody glycosylation in autoimmunity: Sweet biomarker or modulator of disease activity? Nature Reviews Rheumatology. 2017;13(10):621–630.

[20] Irvine, E. B., Alter, G. Understanding the role of antibody glycosylation through the lens of severe viral and bacterial diseases. Glycobiology. 2020;30(4), 241–253.

[21] Um ana, P., Jean-Mairet, J., Moudry, R., Amstutz, H., Bailey, J. E. Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity. Nat Biotechnol. 1999;17(2):176–180.

[22] Krištić, J., Vučković, F., Menni, C., Klarić, L., Keser, T., Beceheli, I., Pučić-Baković, M., et al. Glycans are a novel biomarker of chronological and biological ages. Journals of Gerontology - Series A Biological Sciences and Medical Sciences. 2014;69(7):779–789.

[23] Russell, A. C., Kepka, A., Trbojević-Akmačić, I., Ugrina, I., Song, M., Hui, J., Hunter, M., et al. Increased central adiposity is associated with pro-inflammatory immunoglobulin G N-glycans. Immunobiology. 2019:224(1);110–115.

Report on the Euro-CASE 2020 Conference

The Croatian Academy of Engineering (HATZ), as a long-term member of Euro-CASE, held the international scientific and professional annual conference Euro-CASE 2020 entitled "Dealing with Challenges of the European Energy Transition" on 20 November 2020.

The Euro-CASE 2020 Annual Conference, scheduled for June 2020, was postponed for November this year due to the coronavirus situation in Croatia and worldwide. In order to avoid social contacts, the conference was held on 20 November 2020 using Zoom

application with technical support from the Euro-CASE and ATI Boards.

The annual Euro-CASE conference is the main professional forum organised by the member academies, bringing together leading European academics and experts to discuss the technical aspects of major issues. Through these annual conferences, Euro-CASE aims to maintain a leading role in promoting awareness of excellence in applied sciences and engineering and related issues of key importance to Europe. There is also a desire to ensure that the social effect of technological changes is duly taken into account, taking full consideration of environmental and sustainability aspects.

Sustainable energy production and use are one of the most important challenges of the 21st century. Providing a secure supply of clean, competitive and affordable energy for all presents complex technical, economic, social and political issues that need to be solved for sustainable development. At the Euro-CASE 2020 conference, the above-mentioned challenges were discussed in the context of the European energy transition.

The usual one-day Euro-CASE conference was shortened to a total of 3 hours of lectures and discussions on energy-related topics. The conference was divided into four parts: the introductory part, the first section, the second section and the time when participants could ask their questions in writing under the chat option. After the welcome speeches, the first section entitled "Energy Policies Challenges and Opportunity for Transformation" featured four invited lecturers, while the second section entitled "Implementation, Economic Impact and Challenges" featured five invited lecturers. The conference ended with questions and answers.

The conference was attended by 170 registered participants, which exceeded the expectations of the Academy Management and the Programme-Organising Committee. Out of a total of 23 national academies that make up Euro-CASE, the presidents or deputies of 21 academies attended the conference.

The conference sponsors were Croatian Electrical Power Industry (Hrvatska elektroprivreda d.d.) and the Croatian Vehicle Centre whereas the conference patrons were Croatian Chamber of Commerce and University of Zagreb.

The conference program, presentations of selected lecturers, and recordings of the entire conference can be found on the websites www.euro-case2020.com and www.hatz.hr.

Organisers

Euro-CASE - The European Council of Academies of Applied Sciences, Technologies and Engineering

HATZ - Croatian Academy of Engineering, Zagreb, Croatia

Programme and Organising Committee

V. Andročec, President of the Croatian Academy of Engineering
N. Čavlina, Programme Committee Chair
N. Duić, Secretary of the Department of Power Systems, Croatian Academy of Engineering
S. Krajcar, Board member of the Croatian Academy of Engineering
V. Mrša, Secretary general of the Croatian Academy of Engineering
Z. Terze, Vice President of the Croatian Academy of Engineering

Website

www.euro-case2020.com
www.hatz.hr

Activities of the Croatian Academy of Engineering (HATZ) in 2021
Auspices, Organization/Coorganization of Conferences

Auspices

- Professional Conference "nZEB in practice – Technical solutions for the energetic and static renovation of buildings, February 18 - 19, 2021, Faculty of Architecture of Zagreb, live with partially filled hall depending on epidemiological measures as well as via live streaming;

- International Scientific Conference „1st Croatian Conference on Earthquake Engineering – CroCEE 2021", March 22-24, 2021, Zagreb;

- International Scientific Conference MOTSP (Management of technology – Step to sustainable production), September 8-10, 2021, Poreč;

- 13th International Scientific-Professional Conference WITH FOOD TO HEALTH, September 16-17, 2021, Osijek

Organization/Coorganization of Conferences

- Conference „Croatian Engineer's Day - Engineers as Future Builders, HIS and HATZ, AGG faculties of the University of Zagreb, March 02, 2021, live with partially filled hall depending on epidemiological measures as well as via live streaming;

- Webinar „Hydrogen in Energy Transition", March 04, 2021, Department of Energy Systems of the Croatian Academy of Engineering, conferencing using Zoom platform

- International Scientific-Professional Conference, „Printing & Design 2021", May, 2021