Immune characterization of suicidal behavior in female adolescents

Cherise R. Chin Fatt a, J. David Farrar b, Manish K. Jha a, Abu Minhajuddin c, Taryn Mayes a, Jane A. Foster a, d, Madhu H. Trivedi a, d*

a Center for Depression Research and Clinical Care, Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX, USA
b Department of Immunology, University of Texas Southwestern Medical Center, Dallas, TX, USA
c Department of Population and Data Sciences, University of Texas Southwestern Medical Center, Dallas, TX, USA
d Department of Psychiatry & Behavioral Neurosciences, McMaster University, Hamilton, ON, Canada

ABSTRACT

Background: To address the need to identify potential markers of suicide behavior for adolescents (ages 12–18 years), mass cytometry was used to explore the cellular mechanisms that may underpin immune dysregulation in adolescents with recent suicidal behavior.

Methods: Peripheral blood mononuclear cell (PBMC) samples from 10 female adolescents with a recent suicide attempt and 4 healthy female adolescents were used. A panel of 30 antibodies was analyzed using mass cytometry. We used two complementary approaches to 1) identify the cell types that significantly differed between the two groups, and 2) explore differences in the expression profile of markers on the surface of these cells. Mass cytometry data were investigated using (Center for Disease Control, 2021) Opt-SNE for dimension reduced (Curtin and Heron, 2019), FlowSOM for clustering, and (Bridge et al., 2006) EgdeR and SAM for statistical analyses.

Results: Opt-SNE (a data driven clustering analysis) identified 15 clusters of distinct cell types. From these 15 clusters, cluster 5 (classical monocytes) had statistically lower abundance in suicidal adolescents as compared to healthy controls, whereas cluster 7 (gamma-delta T cells) had statistically higher abundance in suicidal adolescents compared to healthy control. Furthermore, across the 15 cell types, chemokine receptors, CXCR3 (cluster 5) and CXCR5 (clusters 4, 5, 7, and 9), had an elevated expression profile in those with a recent suicide attempt versus healthy controls.

Conclusion: This report demonstrates the utility of high dimensional cell phenotyping in psychiatric disorders and provides preliminary evidence for distinct immune dysfunctions in adolescents with recent suicide attempts as compared to healthy controls.

1. Introduction

Age-adjusted mortality rates of suicide in adolescents and young adults have increased by over 50% in the past two decades, underscoring the urgency to develop novel interventions that can reverse this public health crisis (Center for Disease Control, 2021). In fact, suicide is now the second leading cause of mortality between the ages of 10–24 years in the United States (Curtin and Heron, 2019). While there are a variety of environmental, social, and emotional/behavioral risk factors associated with suicidal behaviors in adolescents (Bridge et al., 2006; King et al., 2019; Zygo et al., 2019), recent research has begun to examine biological risk factors for increased suicidal ideation and behavior (Turecki and Brent, 2016). Dysregulations within the immune system have gained attention for their role in pathophysiology of suicide (Brundin et al., 2017). A number of studies have identified increased proinflammatory cytokines, such as tumor necrosis factor, interleukin (IL)-6, IL-8, and IL-1 β in individuals who have either attempted or died by suicide (Serafini et al., 2013; Janelidze et al., 2015; Black and Miller, 2013).

Early identification of those who are at risk of suicide and clustering into immune phenotypes, then assessing which drugs or treatments work for each phenotype has potential. Identification of specific immune mediators may allow repurposing of immunomodulatory drugs (such as anti-cytokine monoclonal antibodies) as treatments that can mitigate the risk of suicide. Consistent with this notion, our group has previously reported that adolescents with recent suicidal behavior had significantly lower levels of interleukin (IL-4) as compared to healthy controls and youths at risk of depression (Jha et al., 2020a). As one of the physiological functions of IL-4 is to limit the numbers of systemically circulating Th1 cells (Maggi et al., 1992), low levels of IL-4 may increase susceptibility to an autoimmune process (Van Dyken and Locksley, 2013). Consistent with this, previous reports have found evidence for activation of Th1 cells in suicidal depressed patients (Mendlovic et al., 1999), and elevated levels of autoantibodies in depressed patients with...
low levels of circulating IL-4 (Jha et al., 2020b). Taken together, these findings indicate alterations within the adaptive immune system as a novel mechanistic hypothesis linking inflammation and suicidal behavior in patients with depression.

Several studies have found increased pro-inflammatory markers in patients with major depressive disorder (MDD) with high suicidal ideation (SI) as compared to those with MDD and lower SI and healthy controls (O’Donovan et al., 2013a), and in adult suicide attempters, both peripherally (Nassberger and Traskman-Bendz, 1993; Janelidze et al., 2011) and centrally (Lindqvist et al., 2009, 2011). Interestingly, there is also evidence of reduced IL-4 in patients with MDD compared to healthy individuals (Syed et al., 2018). However, the actual effect of a given immune marker is dependent on the context in which it is produced; thus, it is very difficult to infer the true nature of immune regulation from cross-sectional measurement of immune markers. This highlights both the complexity of the immune system, as well as the difficulty in isolating the dysfunction, particularly from cytokines, which are downstream mediators of an immune response. Therefore, characterization of immune tone in adolescents with suicidal behavior will allow us to better understand how immune dysfunction is related to suicidality. This can be accomplished by quantifying the types of cells responsible for engaging cytokines in the immune response.

One method to quantify cells is mass cytometry (CyTOF), a variation of flow cytometry, which is used to quantify cell surface markers (Tanner et al., 2013). For mass cytometry, antibodies are labeled with heavy metal ions unlike flow cytometry which uses fluorochromes. CyTOF takes advantage of quantifying many markers simultaneously on millions of cells to quantify a large number of different cell types (as compared to flow cytometry) (Gadalla et al., 2019). Furthermore, CyTOF also allows for quantifying the expression of multiple immune markers on these distinct immune cell types. In this brief report, in a sample of female adolescents with a recent suicide attempt and healthy controls we used CyTOF to (Center for Disease Control, 2021) determine differences in the abundance of cells between the two groups (Curtin and Heron, 2019), identify differences in the expression profile of markers on the surface of these cells to characterize the cellular mechanisms that may underpin immune dysregulation in adolescents with recent suicidal behavior as compared to healthy controls and (Bridge et al., 2006) to inform future studies utilizing our extensive biobank of participants with depression. Due to the well-recognized sex differences in the immune systems and its association with the pathophysiological mechanisms of depression (Labonté et al., 2017), and the prevalence of suicidality in females, we restricted these analyses to only females to avoid confounding effects related to sex differences.

2. Methods

All study related procedures were completed only after the documentation of informed consent. For participants of age less than 18 years, assent was obtained from the participants in addition to the informed consent from their parents/legal guardians. The studies involving healthy controls and adolescents with recent suicide behavior were approved by the Institutional Review Board (IRB) at the UT Southwestern Medical Center at Dallas.

2.1. Study participants

2.1.1. Adolescents with recent suicide behavior

Adolescents with a previous suicide attempt were recruited from an intensive outpatient program (IOP) at a large metropolitan children’s hospital. The IOP was designed specifically for treatment of adolescents with a recent suicide attempt or severe worsening of suicidal ideation warranting emergency services. Adolescents were recruited to provide baseline and end of treatment blood samples to examine blood-based biomarkers among adolescents at high-risk for suicide. For this pilot analysis, we examined the baseline samples from 10 female adolescents (ages 12–18 years) who had a recent suicide attempt.

2.1.2. Healthy controls

Healthy control adolescents were recruited from an ongoing study, entitled the Texas Resilience Against Depression study (T-RAD). T-RAD is comprised of two inter-linked studies—D2K and RAD—which are designed to be natural history, longitudinal studies that follow participants with current or past diagnosis of a unipolar or bipolar depressive disorder (D2K), or are at risk for depression but not yet suffering from the disease (RAD) (Trivedi et al., 2020). At study enrollment, participants receive comprehensive demographic and psychiatric assessment through a combination of self-report surveys and clinician-rated measures. For this analysis, we focused on adolescents (aged 10–18 years) enrolled in RAD who did not have any psychiatric diagnosis and had low depression (PHQ-9 < 5), anxiety (GAD-7 < 5), and suicidal ideation (CHRT-SR < 2) at baseline. The baseline sample of four aged matched (± 2 years) adolescent females were selected.

2.2. Biospecimen collection and PBMC isolation

Venous blood was drawn from participants and collected in 10 mL EDTA blood tubes. Peripheral blood mononuclear cells (PBMC) were isolated from the whole blood using Lymphprep (Stem Cell Technologies) according to manufacturer’s protocol with minor alterations. Blood layers were separated by centrifugation. PBMCs were collected from the plasma-Lymphprep interface. Concentrations of PBMCs were determined using a hemocytometer. Cells were resuspended in cold fetal bovine serum with 10% dimethyl sulfoxide (DMSO), aliquoted and stored in –80 °C for long-term storage.

2.3. Mass cytometry

A panel of 30 antibodies designed to distinguish a broad range of immune cells was used. Antibodies were purchased in a pre-conjugated form from Fluidigm. The antibodies and reporter isotopes are listed in Supplementary Table 1. Briefly, the PBMC samples were first thawed and stained with anti-CD45 antibody conjugated with 89Y. Then, the PBMCs were mixed and stained with cell surface antibodies for 30 min at room temperature. Subsequently, the samples were permeabilized overnight at 4 °C and stained with cell surface antibodies for 30 min at room temperature. The antibody-labeled samples were washed and incubated in 0.125 nM intercalator-Ir (catalog no. 201192B, Fluidigm) diluted in phosphate-buffered saline (PBS, catalog no. 806544, Sigma-Aldrich) containing 2% formaldehyde and stored at 4 °C until cytometry by time of flight (CyTOF) examination. Before acquisition, the samples were washed with deionized water and then resuspended at a concentration of 1 × 10^6 cells/mL in deionized water containing a 1:20 dilution of EQ Four Element Beads (catalog no. 201078, Fluidigm). The samples were then examined on a Helios mass cytometer (Fluidigm).

2.4. CyTOF data analysis

Data were obtained as .fcs files. The CyTOF data were analyzed with OMIQ data analysis software (www.omiq.ai). Manual gating was applied to focus on CD45+ cells. Opt-SNE algorithm was used on the indicated gated cells (Belkina et al., 2019). For clustering, FlowSOM (an unsupervised clustering technique) with consensus metaclustering was used (Van Gassen et al., 2015).

2.5. Statistics

EdgeR analyses, an implementation of methodology developed by Robinson and Smyth, 2007, 2008, was used to determine whether there are differences in the abundance of cells between healthy control and suicidal participants. Significance Analysis of Microarrays (SAM), a statistical technique used for finding significant features described by a
Cluster 5, participants who had a recent suicide attempt had a lower percent of total cells compared to healthy controls. However, for cluster 7, there were significantly different between participants with a recent suicide attempt and healthy controls. Specifically, classical monocytes were reduced whereas gamma-delta T cells were elevated in adolescents with a recent suicide attempt compared to healthy adolescents.

Using SAM with FDR p < .01, we found 15 markers (Table 1) to have a significantly higher median fluorescence intensity (MFI) in adolescents with recent suicide attempt as compared to healthy controls (FDR p < .01). In general, chemokine receptors CXCR3 (cluster 5) and CXCR5 (clusters 4, 5, 7, and 9) were elevated in adolescents with recent suicide attempt compared to healthy controls.

### 4. Discussion

In this sample of adolescent females (recent suicide attempt and healthy controls), we found that two clusters (clusters 5 and 7) of cells were significantly different in adolescents with recent suicide attempt compared to healthy controls. Specifically, classical monocytes were reduced whereas gamma-delta T cells were elevated in adolescents with a recent suicide attempt as compared to healthy controls. Furthermore, we found multiple cell surface markers on distinct immune cell types, particularly chemokine receptors CXCR5 and CXCR3, had a higher median fluorescence intensity in adolescents with a recent suicide attempt compared to healthy control. In the context of research evidence...
for a role in inflammation in mental health, the present observation of immune activation in adolescent females with a recent suicide attempt demonstrates the importance of considering inflammatory biomarkers in adolescents and young adults at risk of psychiatric illness.

To our knowledge, this is the first effort at using mass cytometry to characterize the immune profile of adolescents with a recent suicide attempt. Previous studies have used a single marker (e.g., CRP) or explored a panel of cytokines and chemokines (Brundin et al., 2017). However, our study extends these previous studies by (Center for Disease Control, 2021) exploring 30 cell surface markers simultaneously (Curtin and Heron, 2019), determining which combination of these 30 markers explains the most differences in the abundance of cells, and (Bridge et al., 2006) identifying differences in the expression profile of markers on the cell surface between healthy controls and adolescents with a recent suicide attempt. Interrogating the immune systems using CyTOF has the potential to discover immunologic changes that are associated with suicidal ideation, and thus guide subsequent treatment (Spitzer and Nolan, 2016).

The findings of this report that adolescents (all female) with recent suicide attempt had lower abundance of classical monocytes and higher abundance of gamma-delta T cells compared to healthy adolescents. These findings line up with prior studies of depression and suicidality which has indicated an excess inflammatory response (Brundin et al., 2017; O’Donovan et al., 2013b; Serafini et al., 2020; Holmes et al., 2018). In addition, elevated levels of CXCR3 and CXCR5 in adolescents with recent suicide attempt suggest there is an increased inflammatory tone. While we deliberately restricted our sample to adolescents and to females, age- and sex-related changes in immune response are well recognized and therefore additional experiments, such as in vitro or in vivo immune challenges, are needed to understand the functional consequences of the altered expression pattern of these immune markers.

There are several limitations of this report. Due to the small sample size, these findings must be considered as preliminary and need to be validated in a larger sample of adolescents with a recent suicide attempt and matched healthy controls. Additionally, the sample was restricted to females only and unknown menstrual cycle information, which limits the interpretation of these findings. The CyTOF was performed only on the cells at initial visit, therefore longitudinal changes in immune response cannot be inferred from these findings. These limitations may be mitigated by large longitudinal studies that enroll participants with recent suicide attempt as well as healthy individuals in the community and characterize their immune profile using mass cytometry and complementary tests such as proteomic, transcriptomic and in vitro immune challenges.

In conclusion, using mass cytometry, we characterized the immune profile of adolescents with a recent suicide attempt, and our preliminary evidence suggests that adolescents with a recent suicide attempt have a decreased number of classical monocytes and increased number of gamma-delta T cells compared to healthy adolescents. These findings as well as future work using mass cytometry, may inform our path forward towards the identification of actionable, objective markers of suicidality. Identification of these markers will significantly advance the field by identifying potential immune targets for treatments to lower suicidality in adolescents.

Declaration of competing interest

Dr. Trivedi has served as a consultant or advisor for Alkermes Inc., Altrino Neuroscience Inc, Axsome Therapeutics, Boehringer Ingelheim, GH Research, GreenLight VitalSigInc Inc, Heading Health, Inc., Janssen Pharmaceutical, Legion Health, Merck Sharp & Dohme Corp., Mind Medicine Inc., Navitor, Neurocrine Biosciences Inc., Noema Pharma AG, Orexo US Inc., Otsuka Canada Pharmaceutical Inc, Otsuka Pharmaceutical Development & Commercialization, Inc. (MDD Section Advisor), SAGE Therapeutics, Signant Health, and Takeda Pharmaceuticals Inc. He receives editorial compensation from Oxford University Press. Dr. Jha has received contract research grants from Acadia Pharmaceuticals and Janssen Research & Development, educational grant to serve as Section Editor of the Psychiatry & Behavioral Health Learning Network, consultancies fees from Eleusis Therapeutics US, Inc, and honoraria for CME presentations from North American Center for Continuing Medical Education and Global Medical Education. Dr. Foster has served on the Scientific Advisory Board for MRM Health NL and has received consulting/speaker fees from Klaire Labs, Takeda Canada and Rothman, Benson, Hedges Inc. Dr. Chin Fatt, Dr. Farrar, Dr. Minhajuddin, and Mrs. Mayes have no conflicts of interest to report.

Acknowledgements

These studies were funded in part by the Elizabeth Jordan Harris Foundation, the W.W. Caruth Jr. Foundation, and the Hersh Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the various funding organizations. We thank the participants, families, staff, and colleagues who made this project possible, and Kathryn Forbes for administrative support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbbih.2022.100499.

References

Belkina, A.C., Ciccololla, C.O., Anno, R., Halpert, R., Spiden, J., Snyder-Cappione, J.E., 2019. Automated optimized parameters for T-distributed stochastic neighbor embedding improve visualization and analysis of large datasets. Nat. Commun. 10, 1–12.

Black, C., Miller, B.J., 2015. Meta-analysis of cytokines and chemokines in suicidality: distinguishing suicidal versus nonsuicidal patients. Biol. Psychiat. 78, 28–37.

Bridge, J.A., Goldstein, T.R., Brest, D.A., 2006. Adolescent suicide and suicidal behavior. J. Child Psychol. Psychiatry Allied Discip. 47, 372–394.

Brundin, L., Bryleva, E.Y., Rajamani, K.T., 2017. Role of inflammation in suicide: from mechanisms to treatment. Neuropsychopharmacology 42, 271–283.

Center for Disease Control, 2021. and Prevention Mortality Multiple Cause-Of-Death, Curtin, S.C., Heron, M., 2019. Death Rates Due to Suicide and Homicide Among Persons Aged 10-24: United States, 2000-2017. NCHS data brief, pp. 1–8, Gadalla, R., Noamani, B., MacLeod, B.L., Dickson, R.J., Guo, M., Xu, W., Lukebele, S., Elskesner, H.J., Irazu, A.A., Hirano, N., 2019. Validation of CyTOF against flow cytometry for immunologic studies and monitoring of human cancer clinical trials. Front. Oncol. 9, 415.

Holes, S.E., Hinz, R., Conen, S., Gregory, C.J., Matthews, J.C., Anton-Rodriguez, J.M., Gerhard, A., Talbot, P.S., 2018. Elevated translocator protein in anterior cingulate in major depression and a role for inflammation in suicidal thinking: a positron emission tomography study. Biol. Psychiat. 83, 61–69.

Janelidze, S., Mattei, D., Westrin, A., Traskman-Bendz, L., Brundin, L., 2011. Cytokine levels in the blood may distinguish suicide attempters from depressed patients. Brain Behav. Immun. 25, 335–339.

Janelidze, S., Suchankova, P., Ekmant, A., Erhardt, S., Sellgren, C., Samuelsson, M., Westrin, A., Minthon, L., Hansson, O., Traskman-Bendz, L., 2015. Low IL-8 is associated with anxiety in suicidal patients: genetic variation and decreased protein levels. Acta Psychiatr. Scand. 131, 269–278.

Jha, M.K., Cai, L., Minhajuddin, A., Fatt, C.C., Furman, J.L., Gadad, B.S., Mason, B.L., Greer, T.L., Hughes, J.L., Xiao, G., 2020a. Dysfunctional adaptive immune response in adolescents and young adults with suicide behavior. Psychooncrodinocinology 111, 104487.

Jha, M.K., Cai, L., Minhajuddin, A., Fatt, C.C., Furman, J.L., Gadad, B.S., Mason, B.L., Greer, T.L., Hughes, J.L., Xiao, G., Emling, G., Kennard, B., Mayes, T., Trivedi, M.H., 2020b. Dysfunctional adaptive immune response in adolescents and young adults with suicide behavior. Psychooncrodinocinology 111, 104487.

King, C.A., Grup-Pheyan, J., Brent, D., Dean, J.M., Webb, M., Bridge, J.A., Spiritio, A., Chernick, L.S., Mahabee-Gittens, E.M., Mistry, R.D., 2019. Predicting 3-month risk for adolescent suicide attempts among pediatric emergency department patients. JCP (J. Child Psychol. Psychiay) 60, 1055–1064.

Lambotte, D., Engmann, O, Purushothaman, I., Menard, C., Wang, J., Tan, C., Scarp, J.R., Moy, G., Loh, Y.H.E., Cahill, M., 2017. Sex-specific transcriptional signatures in human depression. Nat. Med. 23, 1102–1106.

Moy, G., Loh, Y.H.E., Cahill, M., 2017. Sex-specific transcriptional signatures in adolescent suicide attempts among pediatric emergency department patients. JCP (J. Child Psychol. Psychiay) 60, 1055–1064.
Lindqvist, D., Janelidze, S., Erhardt, S., Traskman-Bendz, L., Engstrom, G., Brundin, L., 2011. CSF biomarkers in suicide attempters—a principal component analysis. Acta Psychiatr. Scand. 124, 52–61.

Maggi, E., Parronchi, P., Manetti, R., Simonelli, C., Piccinni, M.P., Rugiu, F.S., De Carli, M., Ricci, M., Romagnani, S., 1992. Reciprocal regulatory effects of IFN-gamma and IL-4 on the in vitro development of human Th1 and Th2 clones. J. Immunol. 148, 2142–2147.

Mendlovic, S., Mozes, E., Eilat, E., Doron, A., Lereya, J., Zakuth, V., Spierer, Z., 1999. Immune activation in non-treated suicidal major depression. Immunol. Lett. 67, 105–108.

Nassberger, L., Traskman-Bendz, L., 1993. Increased soluble interleukin-2 receptor concentrations in suicide attempters. Acta Psychiatr. Scand. 88, 48–52.

O’Donovan, A., Rush, G., Hoatam, G., Hughes, B.M., McCrohan, A., Kelleher, C., O’Farrell, C., Malone, K.M., 2013a. Suicidal ideation is associated with elevated inflammation in patients with major depressive disorder. Depress. Anxiety 30, 307–314.

O’Donovan, A., Rush, G., Hoatam, G., Hughes, B.M., McCrohan, A., Kelleher, C., O’Farrell, C., Malone, K.M., 2013b. Suicidal ideation is associated with elevated inflammation in patients with major depressive disorder. Depress. Anxiety 30, 307–314.

Robinson, M.D., Smyth, G.K., 2007. Moderated statistical tests for assessing differences in tag abundance. Bioinformatics 23, 2881–2887.

Robinson, M.D., Smyth, G.K., 2008. Small-sample estimation of negative binomial dispersion, with applications to SAGE data. Biostatistics 9, 321–332.

Serafini, G., Pompili, M., Serafini, M.E., Stefani, H., Palermo, M., Caryell, W., Girardi, P., 2013. The role of inflammatory cytokines in suicidal behavior: a systematic review. Eur. Neuropsychopharmacol 23, 1672–1686.

Serafini, G., Patrizi, V.M., Aguglia, A., Amerio, A., Sampogna, G., Fiorillo, A., Pompili, M., Amore, M., 2020. A specific inflammatory profile underlying suicide risk?

Systematic review of the main literature findings. Int. J. Environ. Res. Publ. Health 17, 2389.

Spitzer, M.H., Nolan, G.P., 2016. Mass cytometry: single cells, many features. Cell 165, 780–791.

Syed, S.A., Beurel, E., Loevenstein, D.A., Lowell, J.A., Craighead, W.E., Dunlop, B.W., Mayberg, H.S., Dhabhar, F., Dietrich, W.D., Keene, R.W., de Rivero Vaccari, J.P., Nemeroff, C.B., 2018. Defective inflammatory pathways in never-treated depressed patients are associated with poor treatment response. Neuron 99, 914–924 e913.

Tanner, S.D., Baranov, V.I., Oransky, O.I., Bandura, D.R., George, T.C., 2013. An introduction to mass cytometry: fundamentals and applications. Cancer Immunology. Immunotherapy 62, 955–965.

Trivedi, M.H., Chin Fatt, C.R., Jha, M.K., Cooper, C.M., Trombello, J.M., Mason, B.L., Hughes, J., Gadad, B., Czysz, A.H., Toll, R.T., Fuller, A.K.S.S., Mayes, T.L., Minhajuddin, A., Carmody, T., Greer, T.L., 2020. Comprehensive phenotyping of depression disease trajectory and risk: rationale and design of Texas Resilience against Depression study (T-RAD). J. Psychiatr. Res. 122, 22–32.

Turecki, G., Brent, D.A., 2016. Suicide and suicidal behaviour. Lancet 387, 1227–1239.

Tusher, V.G., Tibshirani, R., Chu, G., 2001. Significance analysis of microarrays applied to the ionizing radiation response. Proc. Natl. Acad. Sci. USA 98, 5116–5121.

Van Dyken, S.J., Locksley, R.M., 2013. Interleukin-4 and interleukin-13-mediated alternatively activated macrophages: roles in homeostasis and disease. Annu. Rev. Immunol. 31, 317–343.

Van Gassen, S., Callebaut, R., Van Helden, M.J., Lambrecht, B.N., Demeester, P., Dhaene, T., Saey, Y., 2015. FlowSOM: using self-organizing maps for visualization and interpretation of cytometry data. Cytometry 87, 636–645.

Zygo, M., Pawlowska, B., Potemowska, E., Dreher, P., Kapka-Skrzypczak, L., 2019. Prevalence and selected risk factors of suicidal ideation, suicidal tendencies and suicide attempts in young people aged 13–19 years. Ann. Agric. Environ. Med. 26.