1 ABSTRACT

Background: The supply of human organs available for transplantation remains grossly
inadequate globally. Animal-to-human transplantation, and even more so humanized organ
grown in animals, holds promising results for the start of clinical trials in humans. Very little is
known about the public's willingness to accept different xenotransplantation techniques. This
study aims to determine attitudes towards human-to-human transplantation, animal-to-human
transplantation, and chimera-to-human transplantation in the Belgium context.

8 **Methods**: Secondary school students from Flanders, Belgium, were surveyed between 9 January and June 2019. Socio-demographic details likely to influence participants' attitudes 10 were gathered. Participants were presented with three hypothetical cases (human-to-human 11 transplantation, animal-to-human transplantation, and chimera-to-human transplantation) and 12 asked about their willingness to accept the particular organ. Their risk appetite to accept organs 13 with potentially worse outcomes than the status quo was also evaluated.

Results: 741 complete questionnaires were analyzed. It can be concluded that Flemish secondary school students favored the techniques of xenotransplantation to a lesser extent than allotransplantation; however, most of them did consider it a good solution for organ shortage. Compared to animal-to-human transplantation, chimera-to-human transplantation showed a more positive attitude among the respondents when considered a good organ transplantation solution.

20 Conclusion: Flemish secondary school students favored the techniques of 21 xenotransplantation to a lesser extent than allotransplantation; however, most of them did consider it a good solution for organ shortage. In comparison to animal-to-human 22 23 transplantation, chimera-to-human transplantation showed a more positive attitude among the 24 respondents when considered a good solution for organ transplantation.

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keywords: public perception, organ donation, transplantation, xenotransplantation, chimera,
 Belgium

28 INTRODUCTION

While organs donated from fellow human-beings, i.e., allotransplantation, have been 29 established as the treatment of choice for a variety of (patients with) end-stage organ diseases, 30 31 this source of organs is never enough. In the United States, one person is added to the national 32 waiting list every 9 minutes. At the same time, 30 persons per day are removed from the waiting list because they have become too sick to be transplanted or have died without a lifesaving 33 34 organ.¹ Around 1200 persons are currently waiting for an organ transplant in Belgium, and between 80 and 120 patients die each year while waiting for a lifesaving organ.² Despite 35 various strategies to increase donor availability or the optimization of 'higher-risk,' 'marginal,' 36 or 'extended criteria' organs³, the supply of organs remains grossly inadequate globally. 37

Successful xenotransplantation, i.e., cross-species transplantation, holds the potential of a 38 39 unlimited supply to solve the ever-growing organ shortage. Progress near in 40 xenotransplantation suited for clinical application has generally progressed slowly over the last 50 years, but recent advances in genetically-engineered pigs and new immunosuppressive 41 therapies have revitalized xenotransplantation's potential.⁴ Genome editing tools like TALEN 42 and CRISPR/Cas9 are employed to perform multiple gene knockouts, insert human 43 44 transgenes in pigs and create specific animal organ knockouts to be substituted with humanized organs.⁵ Data on laboratory studies using pig organs in nonhuman primates 45 suggest that animal-to-human transplantation may soon be ready to be tested in human clinical 46 trials.^{5,6} While the Covid-19 pandemic has brought concerns about the possible introduction of 47 zoonotic infections to the foreground for the public, experts are not concerned and remain 48 optimistic about the future of clinical xenotransplantation.⁷ More so, the pandemic has again 49 highlighted the need for public engagement and education on xenotransplantation.⁷ 50

51 All prominent ethical guidance papers and international institutions involved with 52 xenotransplantation regulation have always advocated early public engagement and 53 education. In the 2008 Changsha Communiqué, the World Health Organization considers

54 public education on xenotransplantation's potential risks and benefits as one of its key recommendations.⁸ The International Xenotransplantation Association, supported by the 55 International Transplantation Society, stressed in its 2003 position paper the importance of 56 57 understanding public perception and education to optimize xenotransplantation's clinical success.⁹ The ethical principles of respect for persons, beneficence, and justice necessitate 58 individual and public involvement in all experiments conducted in humans.¹⁰ In the case of 59 xenotransplantation, however, public views on the use of animals and transgenetic animals or 60 61 chimeras could also have significantly influenced the public's acceptance of 62 xenotransplantation trials.

However, a recent systematic review has shown that very little is known about public perceptions of xenotransplantation.¹¹ A study conducted in Japan observed 60% of the public and 84% of researchers supported the creation of human–swine chimeras, and 81% of the public and 92% supported the creation of human–swine chimeric embryos.¹² In a study conducted by Stem Cells Transitional Medicine regarding public support in the US for humananimal chimera research, it was seen that over 44% of participants have some knowledge about chimera research and support the use of chimera organs.¹³

A study focusing on teenagers' views suggested that the acceptance of Xenotransplantation in this specific age group varies considerably depending on the country, with acceptance rates ranging from 40% to 75%. In the same study, university students were seen to have more favorable attitudes varying from 64% to 92%.¹⁴ In a survey conducted among the Spanish Gypsy ethnic population group, 74% of the participant said they would accept an animal organ if they needed it and, 60% favored cadaveric donation.¹⁵

Because local authorities regulate xenotransplantation,^{8,9} there is a need for populationspecific studies. To our knowledge, no public perception study has been done in the Belgium setting yet and, only a few studies worldwide have analyzed public attitudes towards organs from animals that have undergone multiple gene knockouts and supports humanized organs.

Therefore, the aim of this study is to determine attitudes towards human-to-human transplantation, animal-to-human transplantation, and chimera-to-human transplantation in the Belgium context. For this study, we focused on adolescents' attitudes attending the last two years of secondary school in Flanders (Belgium).

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85 METHODS

86 Participants

In the region Flanders (Belgium), secondary education consists of six years (Grades 7-12). 87 Students are divided during the six years into three different tracks: the academic, technical, 88 and vocational track. The academic track (ASO) prepares students for higher education, the 89 90 technical track (TSO) contains theoretical and technical training, and the vocational track (BSO) concentrates upon practical education and prepares students for a specific vocation. 91 92 Participants were recruited through secondary schools using a convenience sampling method. Only students from the last two (academic and technical tracks) or three (vocational track) 93 years were included. Students in these years are typically between the ages of 16 and 19 94 years, Data were collected from February 1, 2019, to June 30, 2019. 95

96 Survey

97 The anonymous written questionnaire (addendum I) first gathered participants' sociodemographic data, such as gender, age, religion, and educational track. The rest of the 98 questionnaire, divided into three sections, focused on human organ transplantation, 99 100 xenotransplantation, and xenotransplantation with organs of human-animal chimeras. Each of the three sections first provided some background on the particular technique, whereafter, we 101 evaluated participants' prior knowledge about the presented methods. To evaluate 102 103 participants' willingness to accept the various techniques, we presented one or more hypothetical cases per technique. Finally, participants were asked to indicate to what extent 104

- they agreed with several statements using a Lickert scale. If more than one question was not
- answered in one or more of the three sections, the questionnaire was excluded.

107 Statistical analysis

The consistency of categorical data was checked with either the Chi-squared test or Fisher's exact test. The Mann-Whitney test was used to detect differences in attitudes for the variables gender and religiosity. The Kruskal Wallis test was used to determine differences in attitude for the variables education, religion, and extent of religiosity. A correlation test was used to examine the correlation between the answers to Xenotransplantation and transplantation statements with organs of human-animal chimeras. A two-sided P-values of <0.01 were considered statistically significant. Statistical analyses were performed using SPSS 26.0.

115 **Ethical approval**

This study protocol and questionnaire were approved by the Research Ethics Committee UZ/KU Leuven (MP007957). Voluntary participation and participant's right to withdraw at any time were discussed with participants before they completed the questionnaire, and researcher contact details were provided to them.

120

121 **RESULTS**

122 **Participants**

123 741 questionnaires were returned. Thirty-two were excluded due to leaving more than 1 124 question blank per section. Therefore, 709 questionnaires were included for further analysis 125 (Table 1). The median age of the respondents was 17 years (range 15-21). The wider than 126 expected age range for the grades included in this study can be ascribed to outlier students 127 that have either progressed faster (2) or slower (13) than normal schooling standards. Outlier 128 aged students were not excluded from the analysis as the form part of the peer-group. It was 129 observed that 43.7% of the participants were male, and 56.0% were female (Table 1). Of all

respondents, 37.5% attended classes in General track (ASO), 38.4% in Vocational track
(BSO), and 23.8% in Technical track (TSO). Approximately half of the participants (50.1%)
said they were religious, of which 88.7% were Roman Catholic, 4.2% Muslim, and 2.8%
Protestant. Of note among the religious respondents is that only 4.8% indicated that they were
actively religious, and 66.8% stated that they did not actively practice their religion.

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136 Attitudes towards human-to-human organ transplantation

The majority (95.3%) had prior knowledge of human-to-human organ transplantation (Table 137 2). Participants with prior knowledge were also more willing to undergo organ transplantation 138 (88.3% versus 65.6%, p=0.001). More than half of the students (56.7%) did not think that 139 receiving an organ would change their personality; however, 16.1% thought it would. (Table 140 3). Fifty-three percent indicated that organ transplantation would influence their view on life 141 and death. Women were more likely to agree with this statement regarding views on life and 142 death than men were (57.6% versus 47.1%, p=0.003). More than three quarter (77.7%) of the 143 144 participants thought organ transplantation is accepted by their family and friends, while only 5.4% thought this would be a problem. 145

Only one-tenth of the respondents (10.3%) stated that they would not psychologically be able to live with someone else's organ, but 61.2% did not foresee prospects of psychological harm in organ transplantation. Participants from the General track (ASO) expressed less concern about the psychological impact (70.9%) compared to respondents from the technical track (TSO) (66.3%) and the vocational track (BSO) (48.9%, p < 0.001).

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152 Attitudes towards animal-to-human transplantation

153 The majority of students (71.1%) had never heard of xenotransplantation before (Table 4).

154 More men than women (34.5% versus 24.2%) already had prior knowledge (p=0.003). Despite

the low level of prior knowledge, 36.1% of participants would accept an animal organ if the risks and results of xenotransplantation were comparable to those of organ transplantation with human organs. Approximately half of the participants (50.4%) doubted the technique, and 13.3% would refuse to accept an animal organ. Technical track (TSO) students had a more positive attitude than General track (ASO), and Vocational track (BSO) students did (p<0.001). Additionally, 53% of the respondents with prior knowledge would accept an animal organ (p<0.001).

162 If there were greater risks and worse results, only 12.8% would be willing to accept an animal 163 organ. Slightly more than half (55.7%) would doubt, and 31.2% would refuse. If a participant 164 would accept an animal organ in case of similar risks and results, 27% of them would also 165 accept in case the risks were higher, and the results were indicated to be worse; 95.1% of the 166 people who refused or were doubting in the first case of human-to-human transplantation, also 167 in the second case of animal-to-human transplantation (p<0.001).

A fourth (25.8%) of the participants thought that receiving an animal organ would change their personality, and 44.1% disagreed (Table 5). Participants from the Vocational track (BSO) were more likely to agree that xenotransplantation would change their personality (33.9%, p<0.001). About half (48.8%) of the participants said they thought they would not feel less human after xenotransplantation, while 27.9% would. One-fifth (20.7%) of the students thought their family or friends would not accept xenotransplantation, while 41.3% thought this would not be a problem.

From the total number of respondents, 32.3% thought xenotransplantation was an animal unfriendly technique, compared to 34.7% of the participants who did not think so. More women than men (39.9% versus 23.2%) indicated that they found this technique animal unfriendly (p<0.001). A fourth of the respondents (24.3%) thought they would not be able to cope living with an animal organ psychologically, and 39.5% thought they would have no problems with it. Around 73.8% were worried about the risks associated with this technique. Only 9.6% did not

worry about it. Finally, 41.9% believed xenotransplantation to be a good solution for the organ
shortage, and 16.2% did not.

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184 Attitudes towards chimera-to-human transplantation

185 The majority of students (87.3%) have not heard of chimeras before (Table 6). More men than 186 women (16.2% versus 9.3%) belonged to the group with prior knowledge (p=0.006). If this 187 procedure had similar results and risks as a transplantation with a human organ, 38.6% would be willing to accept an organ from a chimera. A fifth (19.2%) would refuse, and 41.9% would 188 have doubts. More men than women would accept such an organ (p=0.003). In BSO, there 189 was the least acceptance (p<0.001). More religious than non-religious persons would be willing 190 191 to take an organ of a chimera (44.5% versus 33.1%, p=0.002). Among the respondents with prior knowledge, 20% more people responded positively (p<0.001). 192

In case of worse results and greater risks, 11.4% would be willing to receive a chimera organ, and 36.2% would refuse. Almost twice as many men as women (15.5% versus 8.3%) would still accept a chimera organ if there were more risks (p=0.003). If one would accept an organ of a chimera in case of similar risks and results, 27.7% would also accept one in case of more significant risks and worse results. Of the people who refused or doubted the first case, 98.8% also did in the second case (p<0.001).

Half of the students (50.2%) did not think receiving an organ from a chimera would change their personality (Table 7). Similarly, half of the respondents (50.9%) stated that they would not feel less human after the transplantation. The technique itself was considered animal unfriendly by 39.1%. More women than men (46.3% versus 29.9%) indicated concerns for animals (p<0.001), as well as more non-believers (p=0.008). More than a third of the students (35.7%) thought that one should not combine animal and human DNA, while 28.6% were not concerned. More women than men (43.3% versus 25.9%) were against DNA recombination

- (p<0.001). Non-religious students also took more issue with DNA recombination than religious
 students did (41.3% versus 30.1%, p=0.003). Less than a third (29.3%) thought they could not
 psychologically cope with living with a chimera organ. Approximately 70.5% were concerned
 about the risks associated with human-animal chimera transplantation.
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211 Animal-to-human transplantation versus chimera-to-human transplantation

212 In order to determine whether there was a link between the answers to the statement about allotransplantation and the two different xenotransplantation scenarios, a correlation test was 213 done. For all the statements, a significant correlation was seen with a p-value < 0.001. Thus, 214 people who would not accept a human organ would also not do so for an animal organ in 215 96.7% of the cases if similar results and risks (p<0.001) as well as in 97.8% of the cases if 216 217 worse results and more significant risks (p<0.001)(Table 8). Among those who would refuse a human organ, 92.2% would not want an organ of a chimera if similar results and risks (p<0.001) 218 219 as well as 96.7% if worse results and risks (p=0.009).

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221 DISCUSSION

222 This study was the first published public perception study undertaken in Belgium. Although several studies have been done to determine the public acceptance of xenotransplantation 223 224 and even fewer determining the acceptance of transgenetic organs, a recent systematic review illuminated the difficulty of comparing individual studies to determine factors associated in favor 225 or against xenotransplantation.¹¹ This difficulty is due to these studies' non-standardized 226 approach and results in the decreased ability to draw generalizations from these studies¹¹ 227 including this one. The aim of this study was to determine the attitudes of participants, related 228 229 to the different transplantation techniques. There are some interesting points to consider from 230 this study.

231 Attitudes towards human-to-human organ transplantation

In our study, almost all respondents had previous knowledge of human-to-human organ 232 donation and transplantation, but it is unknown if respondents had direct or familial experience 233 with the matter. A vast majority indicated their willingness to accept the technique if they were 234 waiting for a lifesaving organ and had a 3-month prognosis. A similarly high prior knowledge 235 and acceptance rate is found in the only other contextually comparable study by Coucke et al., 236 who studied Flemish primary health care physicians' attitudes towards organ donation and 237 transplantation.¹⁶ While these studies' comparability is low in terms of respondents' age and 238 level of education, they both align with Belgium's above global average status regarding the 239 240 recruitment of fellow residents to donate their organs posthumous. They, furthermore, aligned with expected public attitudes for a country with a so-called presumed consent organ donation 241 law^{17,18}. 242

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244 Attitudes towards xenotransplantation

245 Both xenotransplantation techniques were in comparison to human-to-human transplantation, mostly unknown techniques to respondents. A degree of scenario transference from human-246 247 to-human transplantation seemed to have influenced the respondents, as a remarkable 36% to 39% were willing to accept the priorly unknown techniques of animal-to-human 248 transplantation or chimera-to-human transplantation. In a study conducted by Febrero et al. in 249 250 the southeast of Spain, it was seen that 44% of the teenagers favored the technique of xenotransplantation.¹⁴ A seemingly higher rate of acceptance of xenotransplantation (51.4%) 251 was found among university students in Italian (51.4%) and Polish (55%) studies^{19,20}, but the 252 lack of more studies amongst teenagers and students limits age comparisons. 253

In our study, we evaluated participants' willingness to accept xenotransplantation if the technique would hold further or more severe risks than human-to-human transplantation. The

256 acceptance rate fell dramatically to around 12%. The questions on risk aversion phrased in a hypothetical scenario are interesting as they may support Persson et al.'s conclusion that it is 257 difficult for a healthy person to judge the various possibilities of treatment in the event of 258 suffering a life-threatening disease, which requires an organ transplant.²¹ Rubaltelli et al. raise 259 260 further caution to the interpretation of these results. They demonstrated that participants' affective reactions would be influenced when human-to-human transplantation and 261 xenotransplantation are compared directly, as supposed to separately.²² One should also 262 263 recognize the potential bias brought about to participants in our study as the specific questions 264 were phrased to focus only on the possible involvement of more risks associated with xenotransplantation. A recent publication shows that xenotransplantation will hold fewer risks 265 or at least different kinds of risks than human-to-human transplantation. The benefits of 266 xenotransplantation, like the absence of a waiting period and superior organ quality, were not 267 explored in this study.23 268

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270 Animal-to-human transplantation versus chimera-to-human transplantation

Overall, our results demonstrated that if the risks were potentially identified similar to both xenotransplantation and organs from chimeras, most of the respondents favored both the techniques (41% for animal-to-human transplantation and 43.5% for chimeras-to-human transplantation). Thirty-two percent of participants agreed the xenotransplantation technique is animal unfriendly, and 39.1% thought human-animal chimeras are animal unfriendly as well. However, approximately the same percentage agreed that it would be a good solution for organ shortage (41.8% for xenotransplantation and 35.2% for human-animal chimeras).

It was examined whether there was a connection with the attitude towards human-to-human
transplantation with the two xenotransplantation techniques. Participants who would not accept
a human organ would also not do so for an animal organ in 96.7% of the cases if similar results

and risks and 97.8% of the cases if worse results and greater risks. Surprisingly, it was also observed that regardless of having the least prior knowledge about human-animal chimeras, there was more acceptance rate observed for the human-animal chimeric organ, especially when participants had to suppose if they were on a waiting list. This observation may be due to a general teenager curiosity and openness to new things, coupled with their tendency to underestimate risks.

We found no other studies that explicitly compared participants' attitudes in the two different 287 scenarios of animal-to-human transplantation and chimera-to-human transplantation. 288 However, in some studies on xenotransplantation, the attitude toward genetic modification in 289 290 donor animals was questioned. In one study among the Korean population, 63% of participants held a positive attitude in this regard.²⁴ In de Bona et al.'s Italian study, 51.4% of students had 291 favorable attitudes towards animal genetic modification for the purpose of transplantation to 292 humans.¹⁹ Besides, several studies have already been conducted on the public's opinion 293 294 concerning research with human-animal chimeras and human-animal embryos. In a poll in 2007 among the British general public, 35% replied they thought hybrid embryos could be 295 created for research; 48% were opposed to this.²⁵ Additionally, an American online survey 296 issued shows 22.6% had opposed chimeric research.¹³ Another survey of the Japanese 297 population showed opposition to research with human-animal chimeras of about 50%.²⁶ 298 Conversely, a year later, another study among the Japanese population appeared in which 299 81% were in favor of the creation of human-pig embryo chimeras, and more than 60% were in 300 favor of the creation of human-pig chimeras.¹² In our study, only 28.6% were in favor of 301 302 transgenetic organ development.

303 Limitations of the study

304 Some limitations were already discussed above, but some further limitation requires 305 mentioning. First, the respondents chosen for the survey were from a particular educational 306 background and age group, which was secondary students. Therefore, our results cannot be

307 generalizable to the general population. Second, the study was conducted prior to the start of 308 the global COVID-19 pandemic with no other major epidemic due to a zoonotic infection that 309 occurred recently in Europe. The general public's understanding of the risks and 310 consequences of zoonotic infections would likely have shifted since the study was conducted.⁷

311

312 CONCLUSION

It can be concluded that Flemish secondary school students favored the techniques of xenotransplantation to a lesser extent than allotransplantation; however, most of them did consider it a good solution for organ shortage. In comparison to animal-to-human transplantation, chimera-to-human transplantation showed a more positive attitude among the respondents when considered a good solution for organ transplantation. As not many previous surveys have been conducted regarding secondary school students' attitudes, we cannot compare the results to other studies except for higher age levels or the general population.

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