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# Olfactory function in congenital cytomegalovirus infection: a prospective study

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80

81  
82 **Abstract**

83  
84 Congenital cytomegalovirus (CMV) infection leads to olfactory bulb lesions in the fetus, yet  
85 little is known about its impact on olfaction after birth. Here, we have assessed in a prospec-  
86 tive study conducted on children in two French hospitals from 2016 to 2019, infection sever-  
87 ity and olfactory performance after congenital CMV infection. Children with congenital CMV  
88 infection aged 3 to 10 years and healthy controls (CTL) matched for age and sex to CMV  
89 children symptomatic at birth (sCMV) were enrolled. Olfactory discrimination was assessed  
90 using mono-odorants and binary mixtures. Data were analyzed for 54 children with PCR-con-  
91 firmed congenital CMV infection, including 34 sCMV (median [IQR] age, 6 [5-8] years; 19  
92 [55.9%] male) and 20 CMV asymptomatic at birth (aCMV, median [IQR] age, 4 [3-6] years;  
93 12 [60.0%] male). sCMV were compared to 34 CTL children. Olfactory scores in CMV-

94 infected children were independent from vestibular deficit and hearing loss. The olfactory  
95 score was efficient to discriminate between CTL and sCMV for children >6 years (area under  
96 the receiver-operating characteristic curve (AUC, 0.85;  $P=0.0006$ ), but not for children <7  
97 years. For children >6 years, the proportion of children with total olfactory score <4 differed  
98 between sCMV and CTL groups (91.2% and 18.7%,  $P < 0.001$ ), but not between aCMV and  
99 age-matched healthy control groups.

100

101 *Conclusion:* Congenital CMV infection is associated with reduced olfactory performance in  
102 children with infection symptoms at birth.

103

104 **Key words:** diagnosis, olfaction, smell, discrimination, odorant mixture, children

105

106

107 **Introduction**

108

109 Cytomegalovirus (CMV) is a herpes type 5 virus that can affect the fetal and neonatal brain  
110 after *in utero* infection [1]. CMV affects 0.5-2% of newborns and is the leading infectious  
111 cause of congenital deafness. Depending on neonatal clinical presentation, children are either  
112 categorized as having a symptomatic (sCMV) (presenting with growth retardation, prema-  
113 turity, jaundice, petechiae, liver and/or hematological anomalies) or asymptomatic (aCMV)  
114 (no clinical sign of infection other than possible hearing loss) infection at birth. Prognostic  
115 factors for neurosensory sequelae comprise gestational age at infection and sCMV [2, 3]. 40-  
116 60% of sCMV and 10-20% of aCMV children will manifest varying degrees of hearing loss,  
117 which can be present at birth or may occur in the first months or years [4]. Although 90% of  
118 clinical presentations are silent at birth, no systematic newborn screening has been established  
119 to identify aCMV children who are at risk of hearing loss. Human CMV has a specific olfac-  
120 tory receptor expressed on olfactory neurons in the olfactory system that may define viral

121 olfactory cell tropism [5]. Congenital CMV exhibits tropism for neural stem cells of the olfac-  
122 tory system of fetuses, thus lesioning the olfactory bulb [6-8]. This infection leads to both ol-  
123 factory and hearing impairments in a mouse model [9]. However, little is known about olfac-  
124 tory dysfunction in CMV-infected children, partly because it is challenging to assess olfaction  
125 in toddlers. Many studies have shown the difficulty to reliably test children under 5 years  
126 [10-13] because of the cognitive and verbal involvement. Discrimination tasks are the most  
127 relevant because they are rapid to perform, unlike threshold tasks, and they are requiring min-  
128 imal cognitive and verbal skills, contrary to identification tasks. New tests based on percep-  
129 tion level could constitute useful tools to address olfaction in children. In this regard, mixture  
130 based olfactory discrimination tests perform better than standard smell tests in adult humans  
131 and in adult and pup animal models [9, 14]. Here, we report the olfactory performance of chil-  
132 dren with a confirmed congenital CMV infection, using a new psychophysical test we have  
133 developed. This test aims at measuring the discrimination of monomolecular odorants from  
134 the Sniffin' test battery [15] and the discrimination of mixture odorants presented in Sniffin'  
135 pens. It is non-invasive and rapid to perform, even in very young children, thus requiring lit-  
136 tle attention and concentration.

137

## 138 **Methods**

139

### 140 **Study Overview and ethical considerations**

141 The main objective of this study was to investigate the association between hearing loss and  
142 olfactory performance in children with a congenital CMV infection followed in Robert Debré  
143 (Paris) and Bicêtre (Le Kremlin- Bicêtre) hospitals, in France. This prospective study is a  
144 nontrial, nondrug study, qualified as exploratory, multicenter, in a paediatric population (Clin-  
145 icalTrials.gov number, NCT02782988). It received ethical approval (N° 3372) from Comité

146 de Protection des Personnes (CPP IDF-3). Children were included in the study after explana-  
147 tion of the study and obtaining of written informed consent from both parents.

148

### 149 **Enrolment Criteria**

150 Children with confirmed congenital CMV, aged 3 to 10 years, were enrolled in this study dur-  
151 ing a standard care visit. Proof of congenital infection was ascertained by positive CMV poly-  
152 merase chain reaction (PCR) in urine and/or blood in the first 3 postnatal weeks, or retrospec-  
153 tive diagnosis for the presence of positive PCR on dried blood spots collected at postnatal day  
154 3 to 7.

155 Exclusion criteria included clinical conditions that may interfere with the study, such as  
156 chronic rhinosinusitis, allergic rhinitis, primary ciliary dyskinesia, Kallmans syndrome or  
157 other neurologic issues that can impact olfaction.

158 CMV infected children were divided into two groups according to neonatal characteristics  
159 consistent with recognized clinical definitions: sCMV and aCMV at birth. Healthy controls  
160 (CTL) matched for age and sex to the sCMV group were enrolled among children consulting  
161 for other ear, nose, throat (ENT) non-rhinological pathologies, anaesthesiology or orthopaedic  
162 appointments. CTL children had no history of congenital infection and presented with transi-  
163 ent evoked otoacoustic emissions <20 dB for each ear.

164

### 165 **Clinical and Radiologic Symptoms**

166 Prenatal and neonatal clinical signs and virological data in favour of congenital CMV infec-  
167 tion were recorded. Postural developmental milestones, vestibular canal and otolithic function  
168 were assessed as previously described [16]. Magnetic resonance imaging of the brain and the  
169 inner ear was performed to assess cerebral lesions (see the Supplemental Information for de-  
170 tails).

171

## 172 **Hearing Evaluation**

173 Children with congenital CMV underwent either objective auditory brainstem response or  
174 subjective behavioral audiometry tests to assess auditory thresholds. Hearing deficit was de-  
175 fined by an auditory threshold of the most affected ear  $\geq 25$ dB. In CTL, normality of hearing  
176 was assessed using evoked otoacoustic emissions.

177

## 178 **Olfactory Evaluation**

179 Olfaction was assessed in a 15-minute session with 18 pen-like odour-dispensing devices  
180 (Sniffin' Sticks, Burghardt, Wedel, Germany) [15]. Two series of 3-odorant discrimination  
181 tasks were performed: the first with simple odorants (monomolecular odorant test), and the  
182 second with binary mixtures of odorants (mixture odorant test). For each task, 3 Sniffin'  
183 Sticks were sequentially presented to the subject, two contained the same odorant and one  
184 contained a different associated odorant. The child was requested to smell each stick and indi-  
185 cate the stick that smells differently (forced choice between three possibilities). A correct or  
186 incorrect answer resulted in a score of 1 or 0, respectively.

187

### 188 ***Monomolecular Odorant Test***

189 The sticks for the first task contained isoamylacetate (one stick) and anethol odorant (two  
190 sticks). The sticks for the second task contained limonene (one stick) and citronellal odorant  
191 (two sticks). The sticks for the third task contained anethol (one stick) and eugenol odorant  
192 (two sticks). The total score for this test ranged from 0 (no correct response) to 3 (all correct  
193 responses). Binary variables were defined using the threshold of 2.

194

### 195 ***Mixture Odorant Test***

196 The sticks for the first task contained a mixture of L-carvone and D-carvone at a 2:8 propor-  
197 tion (one stick) and mixture of L-carvone and D-carvone at a an 8:2 proportion (two sticks).  
198 The sticks for the second task contain a mixture of isoamylacetate and anethol in an 8:2 pro-  
199 portion (one stick) and mix of isoamylacetate and anethol at a 2:8 proportion (two sticks). The  
200 sticks for the third task contain a mixture of anethol and eugenol at an 8:2 proportion (one  
201 stick) and mix of anethol and eugenol at a 2:8 proportion (two sticks). The total score for this  
202 test ranged from 0 (no correct response for the 3 problems) to 3 (correct responses for the 3  
203 problems). Again, binary variables were defined using the threshold of 2.

204

### 205 ***Olfactory Score Calculation***

206 The total olfactory score (TOS) was calculated by adding the monomolecular odorant score to  
207 the mixture score. It ranged from 0 (no correct response for the 6 problems) to 6 (correct re-  
208 sponses for the 6 problems). Binary variables were defined by a total score <4, this threshold  
209 was retained as it corresponds to a majority of incorrect responses.

210

### 211 **Statistical Analysis**

212 Quantitative variables were summarized as median with interquartile range (IQR) and com-  
213 pared across groups using Mann-Whitney non-parametric test. Categorical data were ex-  
214 pressed as percentages and compared between groups using Fisher exact test. The accuracy of  
215 olfactory tests was evaluated by applying data to receiver-operating characteristic (ROC)  
216 curves. To study the associations between children characteristics and olfaction, the Spearman  
217 non-parametric test was used. Statistical analyses were performed using Stata 16 (StataCorp  
218 LLC, Texas, USA) and Prism software (GraphPad, version 9, San Diego, USA), significance  
219 was considered at the level 5%.

220



## 221 **Results**

222

### 223 **Child Characteristics**

224 From May 2016 to December 2019, we recruited 34 sCMV children (median [IQR] age, 6 [5-  
225 8] years; 19 [55.9%] male, Tables 1, S1, S2). We also recruited 34 healthy matched-CTL. As  
226 a supplementary control, we included aCMV children. However, due to absence of CMV  
227 newborn screening in France, enrolment of aCMV was complex, particularly in the 7-10 year  
228 age group, and only 20 aCMV were enrolled (median [IQR] age, 4 [3-6] years (only 5 chil-  
229 dren aged 7-10); 12 [60.0%] male. Thus, we ultimately essentially compared sCMV to CTL  
230 children because we did not reach the targeted number of aCMV children. Fig. 1 shows the  
231 flow chart of the selection process.

232 Among the 54 children with congenital CMV infection, 23 presented hearing or vestibulo-  
233 lar deficit at inclusion. Hearing deficits were reported in 19 children (12 in the sCMV group  
234 and 7 in the aCMV group). Three presented with profound congenital hearing loss at birth (1  
235 in the sCMV group and 2 in the aCMV group).

236

### 237 **Olfactory Performance**

238 Among CTL, both the monomolecular odorant discrimination score and the TOS were  
239 positively correlated with age ( $r=0.42$ ,  $P=0.012$ ; and  $r=0.48$ ,  $P=0.004$ , respectively). In  
240 CTL, TOS was significantly higher in children 7-10 years than in those 3-6 years (median  
241 (IQR): 4.0 [4.0-5.0] and 3.0 [1.0-4.0],  $P=0.002$ ), and in consequence the proportion with a  
242 TOS  $<4$  was significantly lower in CTL 7-10 years than in CTL 3-6 years (18.75% and  
243 66.7%, respectively;  $P=0.007$ , Table 2). Considering the monomolecular odorant discrimina-  
244 tion score, the proportion with a score  $<2$  was significantly lower in controls aged 7-10 years  
245 than in controls aged 3-6 years (6.3% and 55.6%, respectively;  $P=0.003$ ). Considering the

246 mixture odorant discrimination score, the proportion with a score <2 was not different be-  
247 tween CTL aged 7-10 and 3-6 years (37.5% and 61.1%, respectively;  $P=0.30$ ). There was no  
248 association between olfactory scores and sex or with passive smoking.

249 ROC curve analysis revealed that the TOS was efficient to discriminate between CTL  
250 and sCMV for children 7-10 years (area under the ROC curve [AUC]=0.857,  $P=0.0006$ , Fig.  
251 2b), but not for children 3-6 years (AUC=0.519, Fig. 2a). Moreover, for children >6 years, the  
252 mixture score alone was efficient to discriminate between CTL and sCMV (AUC=0.809,  
253  $P=0.003$ , Fig. 2d), but not the monomolecular odorant score (AUC=0.588, Fig. 2c).

254 Overall, the proportion of children with a TOS <4 was significantly higher in the  
255 sCMV group than in the CTL group (73.5% and 44.1%;  $P=0.025$ ). Considering only the  
256 monomolecular odorant discrimination score, there was no difference between the two groups  
257 (Fig. 3b). For the only mixture scores, the proportion of children with a score <2 was signifi-  
258 cantly higher in the sCMV group than in the CTL group (76.5% and 50.0%, respectively,  $P$   
259 =0.043).

260 Stratifying by age, the difference in the proportion of children with a TOS <4 was  
261 highly significant between sCMV and CTL in children 7-10 years of age (91.2% and 18.7%,  
262  $P<0.001$ ), but not in younger children (Fig. 3d).

263 In sCMV children, there was no difference in the TOS between children presenting  
264 with and those without neurological involvement (Fig. S1). There was no difference for the  
265 TOS between sCMV children presenting with hearing loss and those with normal hearing  
266 (Fig. 3e).

267 There was no difference in the proportion of children with a TOS<4 between aCMV,  
268 subset of age-matched sCMV and subset of age-matched CTL children in the 7-10 year age  
269 group as well as in younger children (Fig. S2).

270           There was no difference in the olfactory scores between children who received anti-  
271    ral treatment after CMV detection (n=7) and those without treatment (n=38) (Table 2).

272

## 273    **Discussion**

274

275    This is the first study to assess olfactory function in children with congenital CMV infection  
276    and to report the severity of their altered olfaction ability. The strengths of this study are: i)  
277    PCR-confirmed congenital CMV infection, ii) the documentation of clinical, radiologic and  
278    vestibular symptoms as well as concomitant evaluation of hearing and iii) enrolment of age  
279    and sex-matched CTL.

280           Reduced olfactory score was frequent in congenital CMV infection, occurring in  
281    91.2% of our sCMV patients aged 7-10 years, thus becoming the most frequent sensorineural  
282    deficit in our series. 44.1 % of these patients experience other sensorineural deficits (hearing  
283    loss in 35.3%, vestibular deficit in 38.2%). Conversely, 5 aCMV children aged 7-10 years  
284    demonstrated normal olfaction. The most likely explanation of this observation is the proba-  
285    ble link between olfactory performance and the severity of congenital CMV infection. A re-  
286    cent retrospective study demonstrated that 67% of children with olfactory dysfunction were of  
287    congenital origin, whereas 12% were due to head trauma [13]; the role of congenital infection  
288    being to date unknown, the responsibility of CMV has certainly not yet been evaluated. In  
289    previous studies, loss of smell in infants has been linked to neurodevelopmental disorders, in-  
290    cluding attention deficit/hyperactivity disorders and autism spectrum [10, 17]. Olfaction is  
291    essential for food information, safety, emotion regulation, scaffolds environment perception  
292    and memory, mother-child attachment, and social cognition [18]. However, there is no abso-  
293    lute correlation between neurodevelopmental disorders and olfactory scores, as we do not find  
294    a link between these two in our present series.

295 Olfactory loss can also be observed after other post-viral infections such as rhinovirus,  
296 parainfluenza virus, coronavirus (CoV) 229E and Epstein-Barr virus [19]. Olfactory discrimi-  
297 nation and thresholds were preserved in these latter infections, compared to identification  
298 [20]. Olfactory loss can be an early sign of coronavirus disease 2019 (COVID-19) due to se-  
299 vere acute respiratory syndrome CoV-2; this dysfunction can persist several months and be  
300 associated to an olfactory bulb hypometabolism [21-23]. Fetopathological studies have  
301 demonstrated the presence of CMV in neural stem cells of the olfactory bulb underlining the  
302 specific targeting of the pluripotent cells, rather than olfactory neurons [8].

303 Olfactory scores in our CMV-infected children were independent from age, con-  
304 trasting with CTL children. Improved olfactory performance in healthy children is correlated  
305 with the maturation of the olfactory system with better ability to discriminate with age. This is  
306 not observed in sCMV-infected children, possibly due to the viral targeting of pluripotent  
307 cells [8]. Olfactory scores in our CMV-infected children were independent from hearing loss  
308 or vestibular deficit. These findings contrast with an epidemiological study where a correla-  
309 tion was found between hearing loss and olfactory dysfunction, but infection, in particular  
310 congenital, was not considered as an influential factor [24]. The incidence of cranial neuropa-  
311 thies is higher in patients with post-viral olfactory loss compared to a control population [25];  
312 however, we found no difference for the olfactory score between children presenting neuro-  
313 logical manifestations and those without neurological involvement. These findings suggest  
314 that peripheral (audiovestibular) and central (cerebral) lesions are independent and that neuro-  
315 logical damage did not induce vulnerability to olfactory dysfunction in our sCMV infants.  
316 CMV host entry is probably systemic, associated with macrophage infection [26]. To date,  
317 there is no evidence of CMV spread to the brain through the cribriform plate.

318 Another insight of our study is the greater efficiency of the mixture discrimination  
319 tests in assessing olfactory function in children compared to the mono-odorant testing. While

320 the monomolecular test evaluates the ability to discriminate between two single odorants of  
321 similar concentration, the mixture test is a more difficult perception test with discrimination  
322 of mixtures presenting the same two odorants but in different concentration. Of note, the  
323 odorant mixture discrimination score only discriminates between CTL and CMV from the age  
324 of 7, which strongly limits its use in clinics. The lower discrimination efficacy in younger  
325 children may be due to the subtler olfactory difference between scent pens that children 3-6  
326 may be less attentive to.

327         Limitations of our study include the use of olfactory tests that have not been validated  
328 for children in this version before and a predefined cut-off value that was not based on previ-  
329 ous observations in a control group. The cut-off value first appears in the initial statistical  
330 plan of the study's protocol, that was subject to no change. This cut-off of 4 points to distin-  
331 guish between normosmia and olfactory dysfunction was retained in the initial statistical plan  
332 of this study as it corresponds to a majority of incorrect responses. This cut-off leads to a high  
333 percentage of children in the control group with reduced olfactory function. Another limit of  
334 our study is the small sample size of the human cohort, especially for aCMV patients. Extend-  
335 ing these investigations to a larger group of children, including controls, would allow specify-  
336 ing these first findings. Moreover, this study would benefit from additional approaches to  
337 characterize the olfactory function, by using tests of perception and identification of odorants.

338         In conclusion, this study highlighted the high incidence of olfactory impairment in  
339 children with congenital sCMV infection. As olfactory loss can impact nutrition, social inter-  
340 action, safety and quality of life, early detection of olfactory disorders may lead to olfactory  
341 rehabilitation programs in order to limit neurodevelopmental consequences: recent studies  
342 have demonstrated the importance of olfactory training to improve the olfactory function in  
343 adults [27, 28] and children [29].

344

345 **Supplementary information**

346 **Supplementary methods.**

347 **Table S1.** Congenital CMV diagnostic confirmation of the 54 cytomegalovirus-infected chil-  
348 dren.

349 **Table S2.** Neonatal viral symptom characteristics of the 34 cytomegalovirus-infected children  
350 symptomatic at birth.

351 **Fig. S1.** Olfactory scores in control children and children with congenital cytomegalovirus in-  
352 fection symptomatic at birth, according to neurological involvement.

353

354 **Competing interests**

355 The odorant mixtures are the subject of a patent (WO2017198816A1 published on November  
356 23, 2017) by Institut Pasteur, Centre National de la Recherche Scientifique, and Assistance  
357 Publique–Hôpitaux de Paris on which Drs Lazarini, Lledo, Teissier and Levivien are named  
358 as inventors. Drs Lazarini, Madec, Taieb, Mottez, Lledo and Mr Buivan are employees of In-  
359 stitut Pasteur of Paris that sponsored this research. The remaining authors declare no other  
360 disclosures.

361

362 **References**

363

- 364 1. Coyne CB, Lazear HM (2016) Zika virus - reigniting the TORCH. *Nat Rev Microbiol*  
365 14(11):707-715
- 366 2. Nicloux M, Peterman L, Parodi M, Magny JF (2020) Outcome and management of  
367 newborns with congenital cytomegalovirus infection. *Arch Pediatr* 7(3):160-165

- 368 3. Dahle AJ, Fowler KB, Wright JD, Boppana SB, Britt WJ, Pass RF (2000) Longitudinal  
369 investigation of hearing disorders in children with congenital cytomegalovirus. *J Am*  
370 *Acad Audiol* 11(5):283-290
- 371 4. Goderis J, Keymeulen A, Smets K, et al. (2016) Hearing in Children with Congenital  
372 Cytomegalovirus Infection: Results of a Longitudinal Study. *J Pediatr* 172:110-115.e2
- 373 5. E X, Meraner P, Lu P, et al. (2019) OR14I1 is a receptor for the human cytomegalovirus  
374 pentameric complex and defines viral epithelial cell tropism. *Proc Natl Acad Sci U S A*  
375 116(14):7043-7052.
- 376 6. van Den Pol AN, Mocarski E, Saederup N, et al (1999) Cytomegalovirus cell tropism,  
377 replication, and gene transfer in brain. *J Neurosci* 19(24):10948-10965
- 378 7. Odeberg J, Wolmer N, Falci S, Westgren M, Seiger A, Söderberg-Nauclér C (2006) Hu-  
379 man cytomegalovirus inhibits neuronal differentiation and induces apoptosis in human  
380 neural precursor cells. *J Virol* 80(18):8929-8939
- 381 8. Teissier N, Fallet-Bianco C, Delezoide AL, et al (2014) Cytomegalovirus-induced brain  
382 malformations in fetuses. *J Neuropathol Exp Neurol* 73(2):143-158
- 383 9. Lazarini F, Katsimpardi L, Levivien S, et al (2018) Congenital cytomegalovirus infection  
384 alters olfaction prior to hearing deterioration in mice. *J Neurosci* 38(49):10424-10437
- 385 10. Doty RL (2001) Olfaction. *Annu Rev Psychol* 52:423-452
- 386 11. Hummel T, Bensafi M, Nikolaus J, et al (2007) Olfactory function in children assessed  
387 with psychophysical and electrophysiological techniques. *Behav Brain Res* 180(2):133-  
388 138
- 389 12. Cameron EL, Doty RL (2013) Odor identification testing in children and young adults  
390 using the smell wheel. *Int J Pediatr Otorhinolaryngol* 77(3):346-350
- 391 13. Schriever VA, Hummel T (2020) Etiologies of olfactory dysfunction in a pediatric pop-  
392 ulation: based on a retrospective analysis of data from an outpatient clinic. *Eur Arch*

393 Otorhinolaryngol 277(11):3213-3216

394 14. Hsieh JW, Keller A, Wong M, Jiang RS, Vosshall LB (2017) SMELL-S and SMELL-R:

395 Olfactory tests not influenced by odor-specific insensitivity or prior olfactory experience.

396 Proc Natl Acad Sci U S A 114(43):11275-11284

397 15. Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G (1997) 'Sniffin' sticks': olfactory

398 performance assessed by the combined testing of odor identification, odor discrimination

399 and olfactory threshold. Chem Senses 22(1):39-52

400 16. Maudoux A, Teissier N, Francois M, et al (2020) Vestibular impact of Friedreich ataxia

401 in early onset patients. Cerebellum Ataxias 7:6

402 17. Rozenkrantz L, Zachor D, Heller I, et al (2015) A Mechanistic Link between Olfaction

403 and Autism Spectrum Disorder. Curr Biol 25(14):1904-1910

404 18. Schaal B, Saxton TK, Loos H, et al (2020) Olfaction scaffolds the developing human

405 from neonate to adolescent and beyond. Phil Trans R Soc B 375(1800):20190261

406 19. Suzuki M, Koichi Saito K, Wei-Ping Min, et al (2007) Identification of viruses in patients

407 with postviral olfactory dysfunction. Laryngoscope 117(2):272-277

408 20. Whitcroft KL, Cuevas M, Haehner A, et al (2017) Patterns of olfactory impairment re-

409 flect underlying disease etiology. Laryngoscope 127(2):291-295

410 21. Niklassen AS, Draf J, Huart C, et al (2021) COVID-19: Recovery from Chemosensory

411 Dysfunction. A Multicentre study on Smell and Taste. Laryngoscope 131(5):1095-1100

412 22. de Melo GD, Lazarini F, Levallois S, et al (2021) COVID-19-related anosmia is associ-

413 ated with viral persistence and inflammation in human olfactory epithelium and brain

414 infection in hamsters. Sci Transl Med 13(596):eabf8396

415 23. Guedj E, Lazarini F, Morbelli S, et al (2021) Long COVID and the brain network of

416 Proust's madeleine: targeting the olfactory pathway. Clin Microbiol Infect S1198-

417 743X(21)00238-X



- 418 24. Park JH, Byeon HK, Park KN, et al (2017) Epidemiological association of olfactory dys-  
419 function with hearing loss and dysphonia in the Korean population: A cross-sectional  
420 study. *Medicine (Baltimore)* 96(47):e8890
- 421 25. Jitaroon K, Wangworawut Y, Ma Y, Patel ZM (2020) Evaluation of the Incidence of  
422 Other Cranial Neuropathies in Patients With Postviral Olfactory Loss. *JAMA Otolaryn-*  
423 *gol Head Neck Surg* 146(5):465-470
- 424 26. Farrell HE, Stevenson PG (2019) Cytomegalovirus host entry and spread. *J Gen Virol*  
425 100(4):545-553
- 426 27. Addison AB, Wong B, Ahmed T, et al (2021) Clinical Olfactory Working Group Con-  
427 sensus Statement on the Treatment of Post Infectious Olfactory Dysfunction. *J Allergy*  
428 *Clin Immunol* 147(5):1704-1719
- 429 28. Zhang Y, Mei T, Chen Y, et al (2021) Smell disorders in COVID-19 patients: role of  
430 olfactory training: A protocol for systematic review and meta-analysis. *Medicine (Balti-*  
431 *more)* 100(8):e24862
- 432 29. Mahmut MK, Pieniak M, Resler K, et al (2021) Olfactory training in 8-year-olds increases  
433 odour identification ability: a preliminary study. *Eur J Pediatr* 180(7):2049-2053

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**Table 1.** Characteristics of the children with congenital, PCR-confirmed, CMV infection

Demographics	Total (n=54)	Severity of congenital CMV infection		P Value
		Viral symptoms <sup>a</sup> at birth: sCMV (n=34)	Asymptomatic at birth: aCMV (n=20)	
Boys, No. (%)	31 (57.4)	19 (55.9)	12 (60.0)	0.77
Age at inclusion (years), Median (IQR)	5 (4-8)	6 (5-8)	4 (3-6)	0.27
No. of children with age ≤6 years	34	18	15	0.15
No. of children with age >6	21	16	5	
Confirmed maternal CMV reactivation with neuro sequelae, No.	1	1	0	
Including hearing deficit <sup>b</sup>	0	0	0	
Maternal primary CMV infection <sup>c</sup> , No. (%)	21 (38.9)	9 (26.5)	12 (60.0)	0.020
Including confirmed, No. / suspected, No.	14 / 7	8 / 1	6 / 6	
Timing of CMV congenital infection: known, No. (%)				
Periconceptional or during first trimester (<14 weeks)	12 (57.1)	6 (66.7)	6 (50.0)	
Confirmed	7	6	1	
Including hearing deficit <sup>b</sup>	6	4	2	
Including neurosequelae	6	6	0	
Including intrauterine growth retardation	2	2	0	
Second (≥14 weeks and <28 weeks)	6 (28.6)	1 (10.0)	5 (41.7)	
Confirmed, No. / suspected, No.	4 / 2	0 / 1	4 / 1	
Including hearing deficit <sup>b</sup> : confirmed, No. / suspected, No.	1 / 1	0 / 1	1 / 0	
Neurosequelae: confirmed, No. / suspected, No.	1 / 1	0 / 1	1 / 0	
Third (>28 weeks)	2 (1.0)	1 (10.0)	1 (8.3)	
Confirmed	1	0	1	
Including hearing deficit and neurosequelae	0	0	0	
Antiviral treatment after detection of CMV infection, No./No. of data (%)	7/45 (15.6)	6/30 (20.0)	1/15 (6.7)	0.40
Postuomotor development, No./No. of data (%)				
Head control at age >4 months	5/46 (10.9)	4/30 (13.3)	1/16 (6.3)	0.64
Unsupported sitting at age >9 months	7/51 (13.7)	3/32 (9.4)	4/19 (21.1)	0.40
Unaided walking at age >17 months	12/52 (23.1)	8/33 (24.2)	4/19 (21.1)	0.99
Transcranial Doppler sonography assessment, No. (%)	26 (48.1)	20 (58.8)	6 (30.0)	0.09
Abnormal, No. (%)	8 (30.8)	8 (40.0)	0 (0.0)	0.08

**Table 1.** Characteristics of the children with congenital, PCR-confirmed, CMV infection (continued).

Demographics	Total (n=54)	Severity of congenital CMV infection		P Value
		Viral symptoms <sup>a</sup> at birth: sCMV (n=34)	Asymptomatic at birth: aCMV (n=20)	
Cerebral computed tomography and MRI assessment, No. (%)	35 (64.8)	23 (67.6)	12 (60.0)	0.52
Abnormal, No. (%)	25 (71.4)	20 (87.0)	5 (41.7)	0.024
including microcephaly	1	1	0	
Intracerebral calcifications	2	1	1	
Hyperintense signals in the white matter	17	13	4	
Ventricular dilations	5	5	0	
Ischemic lesions	1	1	0	
Olfactory bulb agenesis	1	1	0	
Cerebellar abnormalities	3	3	0	
Sensorineuro and neurocognitive disorders at inclusion, No. (%)	30 (55.6)	21 (38.9)	9 (16.7)	0.18
CNS only	7	6	1	
PNS only	4	-	4	
Including hearing loss <sup>b</sup>	3	-	3	
Mixed	19	15	4	
Including hearing loss <sup>b</sup>	16	12	4	
Behavioural disorders	5	4	1	
Hyperactivity	4	3	1	
Autism	1	1	0	
Hearing <sup>b</sup> or vestibular dysfunctions at inclusion, No. (%)	23 (42.6)	15 (44.1)	8 (40.0)	>0.99
Hearing deficit <sup>b</sup> at birth	3	1	2	
including boys, No.	3	1	2	
Hearing deficit <sup>b</sup> at enrolment, No. (%)	19 (35.2)	12 (35.3)	7 (33.3)	0.61
in boys, No.	13	8	5	0.64
Bilateral symmetric	1	3	1	
Bilateral asymmetric (10dB)	3	2	1	
Unilateral	6	4	2	
Auditory threshold of the most affected ear <sup>d</sup> - dB, median (IQR)		100 (60-100)	70 (40-100)	0.60
Auditory threshold of the least affected ear <sup>d</sup> - dB, median (IQR)		15 (10-35)	20 (15-40)	0.71
Profound and severe hearing loss: No. (%) with auditory threshold $\geq$ 61dB	16 (29.6)	10 (29.4)	6 (30.0)	

**Table 1.** Characteristics of the children with congenital, PCR-confirmed, CMV infection (continued).

Demographics	Severity of congenital CMV infection			P Value
	Total (n=54)	Viral symptoms <sup>a</sup> at birth: sCMV (n=34)	Asymptomatic at birth: aCMV (n=20)	
Cochlear implants <sup>e</sup> , No. (%)	9 (16.7)	5 (14.7)	4 (20.0)	
Bilateral implants, No. (%)	5 (9.3)	2 (5.9)	3 (25)	
Vestibular deficit, No. (%)	20 (37.0)	13 (38.2)	7 (35.0)	>0.99
Complete and bilateral (areflexia)	2	1	1	
Partial and bilateral	5	2	3	
Canalar disorders alone	-	-	-	
Otolithic disorders alone	-	-	-	
Mixed disorders	5	2	3	
Partial and Unilateral	13	10	3	
Canalar disorders alone	1	1	-	
Otolithic disorders alone	-	-	-	
Mixed disorders	12	9	3	
Severity scale for vestibular dysfunction				
0, No. (%)	35 (64.8)	21 (38.9)	14 (25.9)	
1 (unilateral), No. (%)	13 (24.1)	10 (18.5)	3 (5.6)	
2 (bilateral), No. (%)	6 (11.1)	3 (5.6)	3 (5.6)	
Both hearing and vestibular deficit, No. (%)	15 (27.8)	10 (18.5)	5 (9.26)	
Including bilateral symmetric hearing loss	1	1	0	
Including profound and severe hearing loss (>61 dB)	13	8	5	
Including bilateral vestibular dysfunction	5	2	3	
Including both bilateral hearing and vestibular dysfunction	1	1	0	

<sup>a</sup>Viral symptoms at birth: one at least of the following neonatal symptoms: intrauterine growth retardation, prematurity, petechiae, organomegaly, icteriae, thrombocytopenia;

<sup>b</sup>Maternal primary infection: Cases with high IgG avidity in the first trimester were considered as non-primary infections. Cases with seroconversion and/or positive IgG positive IgM, and low or intermediate IgG avidity in first trimester were considered as primary infections in the first trimester. Cases with negative IgG and IgM levels in the first trimester (at 12 to 14 weeks) were classified in either the second or third trimester groups, depending on the date of seroconversion.

<sup>c</sup>Hearing deficit: auditory threshold of the most affected ear  $\geq 25$ dB.

<sup>d</sup>In those with hearing deficit and no implant.

<sup>e</sup>Cochlear implants were usually performed in the early infancy, before 6.

**Table 2.** Olfactory scores by characteristics in controls and CMV-infected children

Variable	No. (%)		
	Monomolecular odorant discrimination Score <2	Mixture odorant discrimination Score <2	Total olfactory Score <4
<b>Controls (n=34)</b>			
Age group, y			
≤6 years (n=18)	10 (55.6)	11 (61.1)	12 (66.7)
>6 years (n=16)	1 (6.3)	6 (37.5)	3 (18.75)
<i>P</i> Value	0.003	0.30	0.007
Sex			
Girls (n=15)	5 (33.3)	6 (40.0)	6 (40.0)
Boys (n=19)	6 (31.6)	11 (57.9)	11 (57.9)
<i>P</i> Value	>0.999	0.49	0.49
Passive smoking			
Yes (n=8)	2 (25.0)	2 (25.0%)	1 (12.5)
No (n=26)	9 (34.6)	15 (57.7%)	14 (53.9)
<i>P</i> Value	>0.999	0.22	0.053
<b>CMV-infected children (n=54)</b>			
Age group, y			
≤6 years (n=34)	20 (60.6)	19 (57.6)	22 (66.7)
>6 years (n=21)	5 (23.8)	18 (85.7)	15 (71.4)
<i>P</i> Value	0.012	0.038	0.772
Sex			
Girls (n=23)	11 (47.8)	19 (82.6)	20 (86.7)
Boys (n=31)	14 (45.2)	18 (58.1)	17 (54.8)
<i>P</i> Value	>0.999	0.077	0.017
Passive smoking			
Yes (n=10)	4 (40.0)	7 (70.0)	6 (60.0)
No (n=44)	24 (54.6)	30 (68.2)	31 (70.5)
<i>P</i> Value	0.49	>0.999	0.71
Antiviral treatment after CMV detection			
Yes (n=7)	3 (42.9)	6 (85.7)	7 (100.0)
No (n=38)	18 (47.4)	25 (65.8)	23 (60.5)
<i>P</i> Value	>0.999	0.407	0.077
Hearing deficit			
Yes (n=19)	11 (57.9)	14 (73.7)	14 (73.7)
No (n=35)	16 (45.7)	26 (74.3)	25 (71.4)
<i>P</i> Value	0.57	>0.999	>0.999
Vestibular deficit			
Yes (n=20)	9 (45.0)	1 (75.0)	13 (65.0)
No (n=31)	13 (41.9)	19 (61.3)	20 (66.7)
<i>P</i> Value	>0.999	0.37	>0.999

Olfactory score is the sum of monomolecular and mixture odorant discriminations. Passive smoking is defined by exposition to more than a tobacco pack per day; Hearing deficit is defined by auditory threshold of the most affected ear  $\geq 25$ dB; Controls had normal hearing (inclusion criterion).

## Legends of the figures

**Fig. 1.** Enrolment in the INFECSMELL-CLIN study. This study was performed between May 2016 and December 2019 in two hospital centers in Paris, France.

**Fig. 2.** ROC curves for the discrimination of children with congenital cytomegalovirus infection and controls using the olfactory scores. Panels a-d show the ROC curves for the discrimination of sCMV and matched controls between 3-6 years (a) and 7-10 years (b-d) using the olfactory score (a, b), the monomolecular odorant score (c) and the mixture score (d). N=34 sCMV; N=34 CTL.

**Fig. 3.** Olfactory scores in children with congenital cytomegalovirus infection and controls. Panels a-e show the total olfactory score (a, d, e), the monomolecular odorant (b) and mixture (c) scores. Box and whiskers showing median, 10 percentile, 25 percentile, 75 percentile, and 90 percentile in bar graphs.  $P < 0.05$  are shown. N=54 CMV including 34 sCMV and 20 aCMV. N=34 CTL.