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Konzo risk factors, determinants and etiopathogenesis: what is new? A systematic review

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HIGHLIGHTS

- Dietary cyanide poisoning and protein malnutrition are major risk factors of konzo.
- Thiocyanate may underestimate the level of cyanide poisoning in konzo patients.
- Konzo etiopathogenesis probably results from an interplay of multiple factors.
- Geo-environmental and psycho-emotional factors are plausible determinants of konzo.
- New insights on the etiology of konzo are raised, but more are still needed.

ABSTRACT

Konzo is a toxico-nutritional upper motor neuron disease causing a spastic paraparesis in schoolchildren and childbearing women in some African countries. Almost a century since the first description of konzo, its underlying etiopathogenic mechanisms and causative agent remain unknown. This paper aims at refreshing the current knowledge of konzo determinants and pathogenesis in order to enlighten potential new research and management perspectives. Literature research was performed in PubMed and Web of Science databases according to the PRISMA methodology. Available data show that cassava-derived cyanide poisoning and protein malnutrition constitute two well-documented risk factors of konzo. However, observational studies have failed to demonstrate the causal relationship between konzo and cyanide poisoning. Thiocyanate, the current marker of choice of cyanide exposure, may underestimate the actual level of cyanide poisoning in konzo patients as a larger amount of cyanide is detoxified via other unusual pathways in the context of protein malnutrition characterizing these patients. Furthermore, the appearance of konzo may be the consequence of the interplay of several factors including cyanide metabolites, nutritional deficiencies, psycho-emotional and geo-environmental factors, resulting in pathophysiologic phenomena such as excitotoxicity or oxidative stress, responsible for neuronal damage that takes place at sparse cellular and/or subcellular levels.

Keywords: Konzo; motor neuron disease; cassava; cyanide intoxication; malnutrition.

1 1. Introduction

Konzo is a neurological disease characterized by a sudden onset of symmetrical, non-progressive 2 and irreversible spastic paraparesis due to selective upper motoneuron damage (World Health 3 Organization, 1996). It mostly affects children from 2 years of age and childbearing women 4 (Chabwine et al., 2011; Siddiqi et al., 2020; Tylleskär et al., 1995), causing gait difficulties. The 5 typical spastic gait rapidly develops within a few hours up to one week (Ministry of health 6 Mozambique, 1984; Tylleskär et al., 1995; World Health Organization, 1996). Konzo is an 7 exclusively African disease involving less than ten countries: the Democratic Republic of the 8 9 Congo (DRC) (Banea et al., 2015b, 1997b, 1992a; Carton et al., 1986; Chabwine et al., 2011; Lucasse, 1952; Okitundu et al., 2014; Tylleskar et al., 1991), Mozambique (Cliff et al., 2011, 1997; 10 11 Ernesto et al., 2002; Essers et al., 1992; Ministry of health Mozambique, 1984; Nhassico et al., 2016), Tanzania (Howlett et al., 1990; Mlingi et al., 2011, 1991), Cameroon (Ciglenečki et al., 12 13 2011; Lantum, 1998), Angola (Allen, 2010), Central African Republic (Mbelesso et al., 2009; 14 Tylleskar et al., 1994) and Zambia (Kasonde, 2015; Siddiqi et al., 2020). More than a half of all konzo cases have been reported from the DRC (Bonmarin et al., 2002), and the country contains 15 zones at the highest risk. Occurrence of konzo epidemics peaks during drought (mostly between 16 June and September), famine or war (Banea et al., 2015a, 1992a; Chabwine et al., 2011; Tylleskar 17 et al., 1991). In some countries, sporadic cases occur outside epidemic periods (Banea et al., 1997a; 18 Chabwine et al., 2011; Ernesto et al., 2002; Tylleskar et al., 1994). 19

20 Konzo was reported for the first time in 1938 by Trolli et al. in the Congolese province of Bandundu (Carton et al., 1986; Lucasse, 1952), although the anthropological literature suggests that it was 21 known from the late 1800s (Kashala-Abotnes et al., 2018). The prevalence of konzo is most 22 probably underestimated (report of 6788 cases in 2009 (Nzwalo and Cliff, 2011), while the 23 24 National Program of Nutrition gave an estimate of 100,000 in the DRC (Cliff, 2010; Diasolua Ngudi, 2005)). Konzo remains a poorly known disease that cannot be properly diagnosed by most 25 local health practitioners (Nzwalo and Cliff, 2011). Furthermore, affected communities are poor, 26 live in remote areas, have a low education level, and hold several cultural and religious beliefs 27 28 regarding konzo. As a result, patients with konzo do not resort to health structures (Chabwine et al., 2011; Nzwalo and Cliff, 2011). 29

Although the number of konzo patients seems low worldwide, its prevalence in locally affected areas can be as high as 20 % (Boivin et al., 2013; Rosling et al., 1988). Moreover, konzo irreversibly handicaps an active portion of the population, yielding a heavy economic and social burden on the community. Thus, konzo constitutes a major public health problem and one of the most prevalent neurological diseases in affected areas (Tylleskar et al., 1991). However, despite major impact on
local communities, konzo can be considered as a neglected disease because it is overlooked most
of the time and ignored by local and national health structures, as well as by the scientific
community, while diseased people do not receive appropriate health care and are stigmatized for
being handicapped. Noticeably, konzo is not even mentioned in the World Health Organization
(WHO) list of neglected diseases (World Health Organization, 2010).

Konzo occurs in food-deprived communities exposed to dietary cyanide from insufficiently 40 processed toxic cassava (Manihot esculenta) (Tylleskar et al., 1991). Cassava is a starchy, tuberous 41 root which constitutes a staple food and a major caloric source for about 800 million people mainly 42 43 residing in tropical regions (Food and Agriculture Organization, 2013). However, cassava protein 44 content, especially sulfur amino acids (SAA) such as methionine and cysteine, is very low (Montagnac et al., 2009a). Thus, populations relying on an exclusive and monotonous cassava-45 based diet (such as in konzo-affected areas) run a high risk of suffering from protein malnutrition 46 (Chabwine et al., 2011). Indeed, protein malnutrition and cassava-derived cyanide poisoning are 47 48 well documented as konzo risk factors since the very first reports (Cliff et al., 1985; Ministry of health Mozambique, 1984), whereas causal factor and underlying etiopathogenic mechanisms 49 50 remain unknown (Kassa et al., 2011; Siddigi et al., 2020).

In the 1980's until late 1990's, several studies were conducted to identify the etiology of konzo and 51 underlying mechanisms. Subsequently, many hypotheses were proposed, including infections such 52 as the human immunodeficiency virus (HIV) and the human T-lymphotropic virus (HTLV), 53 vitamin deficiencies, toxic agents (cyanide and its metabolites), environmental and genetic factors, 54 55 etc. (Kashala-Abotnes et al., 2018; Oluwole, 2015). However, all failed to be confirmed as causal agents of konzo. On the other hand, none of the studied experimental animal models succeeded in 56 57 reproducing the clinical picture of konzo. New theories have recently emerged (Kashala-Abotnes et al., 2018; Tshala-katumbay et al., 2016) but have not yet been integrated in the current conceptual 58 59 frame of thinking regarding the pathogeny of konzo.

Thus, the aim of this paper was to refresh the current knowledge of konzo determinants and pathogenesis in order to enlighten potential new research and management perspectives. In particular, the implications of cyanide metabolites, nutritional deficiencies, and contribution of psycho-emotional and non-climatic geo-environmental factors in the appearance of konzo are discussed.

65 **2. Methods**

66 2.1 Study design

We performed a systematic review of the literature following the Preferred Reporting Items for
Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

69 2.2. Data sources and search strategies

Literature search was conducted in PubMed and Web of Science databases until June 30, 2020.
Relevant publications found among references of the selected articles were also manually searched
for additional studies. We searched for all references containing the key word "konzo" in their title
or abstract, and from the retrieved results, we selected both observational and experimental studies
concerning the risk factors, the etiologies or the pathogenic mechanisms involved in konzo.

Two authors (MB and FN) independently screened the titles and abstracts of all the articles from the search results to determine the articles for full-text review and applied protocol inclusion and exclusion criteria to the full-text publications. Any disagreements were resolved by consensus.

78 The articles selection process is summarized in Figure 1.

79

[Figure 1]

80 3. Results and discussion

81 **3.1.** Clinical presentation of konzo

Konzo presents as a symmetric spastic paraparesis (or a tetraparesis in severely affected patients) 82 83 of abrupt onset and non-progressive course (World Health Organization, 1996). It usually occurs in healthy children, who suddenly present difficulties to wake up and to walk in the morning 84 85 (Ministry of health Mozambique, 1984). Sometimes, especially in childbearing women, the disease may appear after a physical effort such as a long walk or hard work (Ministry of health 86 87 Mozambique, 1984; World Health Organization, 1996). The paraparesis is immediately spastic, with no initial phase of flaccidity and reaches its maximal intensity in less than one day in about 88 89 90 % of patients (World Health Organization, 1996). On neurological examination, patients typically have a spastic (scissor) gait, associated with bilaterally exaggerated knee and/or ankle 90 91 reflexes and sometimes bilateral ankle clonus (Banea et al., 2016; Tshala-katumbay and Spencer, 2007), all signs of a bilateral pyramidal syndrome. On this basis, the WHO has defined simple 92 clinical diagnostic criteria, using the presence of 4 of the following elements: a visible symmetric 93

spastic abnormality of gait while walking or running; a history of onset of less than 1 week followed 94 95 by a non-progressive course in a formerly healthy person; bilaterally exaggerated knee or ankle jerks without signs of disease of the spine, and the absence of consumption of grass pea (World 96 97 Health Organization, 1996). The absence of symptoms related to a spinal cord lesion indicates damage to the upper motor neuron (Ali Ekangu et al., 2015; Mwanza et al., 2003; D Tshala-98 99 Katumbay et al., 2002), even though existing data have failed to localize the exact site of the lesion (Kashala-Abotnes et al., 2018). The spastic paresis sometimes extends to all four limbs, resulting 100 101 in a tetrapyramidal syndrome (Kassa et al., 2011; Nzwalo and Cliff, 2011; World Health Organization, 1996). 102

103 Although in most cases, symptoms associated with konzo appear suddenly and are limited to spastic 104 paraparesis, without apparent announcing symptoms, some patients report general symptoms such as pain (especially in the legs), headache, dizziness, vomiting and rarely fever (Carton et al., 1986; 105 106 Ministry of health Mozambique, 1984), a few days before the onset of the paraparesis. In other cases, the motor deficit is associated with other signs such as pseudobulbar dysarthria, visual 107 108 complaints and vestibular or sensory symptoms, thereby overlapping with tropical ataxic 109 neuropathy (TAN), another disease associated with consumption of toxic cassava, but with a different geographical distribution (Nigeria, Sierra Leonne, India and Cuba) (Adamolekun, 2011; 110 111 Oluwole, 2015; Osuntokun et al., 1968), except for one report in Tanzania 50 years ago (Makene 112 and Wilson, 1972). Finally, recent studies have shown an association between konzo and impaired 113 neurocognition, especially in children (Boivin et al., 2017, 2013; Bumoko et al., 2014; Luwa E-Andjafono Daniel Okitundu et al., 2018; Rivadeneyra-Domínguez and Rodríguez-Landa, 2020), 114 115 although it is not yet clear whether the underlying mechanisms are related to cassava toxicity or other coexisting factors. 116

117 Differential diagnosis in the presence of typical symptoms of konzo can be made with other diseases of the central nervous system causing predominant bilateral pyramidal syndrome. For this 118 119 reason, spinal cord macroscopic lesions should primarily be excluded because their presence conceptually excludes konzo (Tshala-katumbay and Spencer, 2007; World Health Organization, 120 121 1996). This probably also applies to brain lesions affecting motor pathways, but available data on 122 brain structure and function of konzo patients are limited (D Tshala-Katumbay et al., 2002; Desire 123 Tshala-Katumbay et al., 2002; Tshala-Katumbay et al., 2001, 2000; Tshala-katumbay and Spencer, 2007). Also, consumption of grass pea has to be thoroughly searched for, as it is associated with 124 neurolathyrism, a disease with similar symptoms. (Tshala-katumbay and Spencer, 2007; World 125 Health Organization, 1996). The latter is a toxico-nutritional disease associated with a selective 126

damage of the upper motor neuron, caused by the prolonged ingestion of grass pea (Lathyrus 127 128 sativus) in a context of protein malnutrition (Ngudi et al., 2012). Even if the main symptoms of neurolathyrism and konzo resemble each other (i.e. spastic paraparesis, appearance in food-129 deprived conditions), some epidemiological and clinical characteristics allow differentiation 130 between them: while konzo mainly occurs in children and childbearing women, neurolathyrism 131 mostly affects male adults (Oluwole, 2015; Spencer et al., 1986). Furthermore, these 2 diseases do 132 not occur in the same geographic areas (Ethiopia, Spain, India, Bangladesh for neurolathyrism 133 134 (Tshala-katumbay and Spencer, 2007; Woldeamanuel et al., 2012)), and grass pea, the drought tolerant legume involved in the occurrence of neurolathyrism, is neither cultivated nor consumed 135 136 in regions where konzo cases are reported. Neurological symptoms occur in a less abrupt manner in neurolathyrism (with weakness appearing in most cases after 10-15 days, sometimes up to 3 137 138 months, following prodromal symptoms of myalgia, cramps and stiffness(Getahun et al., 2002; Woldeamanuel et al., 2012)). In konzo, 90 % of patients have an onset of weakness in less than one 139 day (World Health Organization, 1996)). 140

141 In summary, diagnosis of konzo is easy, based on well-defined clinical criteria (spastic paraparesis) in the presence of the two risk factors, i.e., consumption of toxic cassava products and protein 142 malnutrition. However, a careful differential diagnosis has to be made to search for (eventually 143 treatable) central nervous system lesions and other toxico-nutritional diseases related to cassava 144 145 (TAN) or grass pea consumption (neurolathyrism). Lesion should be understood here as 146 "identifiable by currently available tools or published reports", since cellular or subcellular damages below sensitivity of standard diagnostic techniques cannot be excluded (Kashala-Abotnes 147 et al., 2018; Sreeja et al., 2003). Other differential diagnoses possibly involved in or associated 148 with konzo (e.g., vitamin deficiencies) will be more appropriately discussed in the section 3.5. 149 below. 150

151 **3.2.** The lesion site in konzo

It is now well established that the disease mainly affects the upper motoneuron (Donaghy, 1999). 152 However, the exact site of the neuronal damage is still unknown, as the few structural 153 investigations performed in the central nervous system (two autopsies by Trolli in 1937 (Tshala-154 katumbay and Spencer, 2007; Tylleskar, 1994) and two magnetic resonance imaging in 155 1991(Tylleskar et al., 1993)) of konzo patients failed to show any macroscopic lesion. This is 156 157 consistent with findings in most motor neuron diseases (MND), such as amyotrophic lateral sclerosis, primary lateral sclerosis, or progressive muscular atrophy. The diagnosis of these diseases 158 is currently mainly based on a correct clinical examination searching for signs of the motor neuron 159

involvement, and exclusion of other neurological conditions that might mimic motor neuron 160 161 dysfunction by neuroimaging (which is usually normal in MND) (Kassubek et al., 2012; Sawalha et al., 2019). Although no single test can confirm the diagnosis of motor neuron disease, 162 electrophysiological investigations may be useful to support the diagnosis of MND (Rocha et al., 163 2005). Electrophysiological measurements (especially sensorimotor and visual evoked potentials) 164 165 in konzo patients have confirmed motor pathways involvement, but additionally suggested optic nerve lesion and, to a lesser extent, sensory pathways dysfunction (Ali Ekangu et al., 2015; 166 167 Kashala-Abotnes et al., 2018; Mwanza et al., 2003; D Tshala-Katumbay et al., 2002).

168 The pathogenic processes underlying MND are not yet fully elucidated (Shaw, 2005). Several 169 mechanisms (such as excitotoxicity, oxidative stress, proteins alterations, or genetic factors) are 170 involved in the neurodegeneration observed in MND (Cluskey and Ramsden, 2001; Rocha et al., 171 2005; Shaw, 2005). A study by Kassa et al. has reported alterations in the expression of proteins 172 involved in many cellular processes (such as the maintenance of the cytoskeleton integrity, control of vesicular trafficking, or regulation of oxidative mechanisms) in rats receiving one daily injection 173 174 of linamarin (50-200 mg/kg body weight) or sodium cyanate (200 mg/kg body weight) (Kassa et al., 2011). Additionally, these alterations were further emphasized in rats on a SAA-deficient diet. 175 176 These observations suggest that the motor neuron degeneration induced by konzo may result from neuronal damage at cellular or molecular levels. 177

178 **3.3.** Dietary cyanide poisoning and its detoxification pathways

179 In all communities where konzo is reported, people rely on cassava roots as the main staple food, particularly during periods of drought, famine, war or other humanitarian disasters (Chabwine et 180 181 al., 2011). During these hard times the bitter varieties of cassava are preferred because they withstand harsh climatic conditions and pestilence (Imakumbili et al., 2019; Kimani, 2011) and 182 have a better yield (Cliff et al., 1997; Howlett et al., 1990; Imakumbili et al., 2019; Tylleskär et al., 183 1995). The bitter taste is due to a high content of cyanogenic glucosides, mainly linamarin (about 184 95 %) and a small quantity of lotaustralin (a methyl-linamarin species) (Montagnac et al., 2009b). 185 Because of their high cyanogenic compounds content and unpleasant taste, the bitter varieties of 186 cassava have to be adequately processed to ensure safety and acceptable taste before consumption 187 188 (Padmaja, 1995; Szabo et al., 2010).

189 Most traditional processing methods used in communities consuming cassava, including water-190 soaking or heap-fermentation of cassava roots, proved to be efficient (Adamolekun, 2011) in 191 reducing cyanogen rates below the toxic threshold of 10 ppm (= mg of total cyanide per kg of 192 cassava) established by the Food and Agriculture Organization (FAO) and the WHO (Joint 193 FAO/WHO Food Standards Programme. Codex Alimentarius Commission, 2009). During the 194 processing, root integrity is altered, allowing linamarase, an enzyme contained in the root cell 195 walls, to hydrolyze linamarin into glucose and acetone cyanohydrin (Adamolekun, 2011). In 196 specific conditions of temperature (>30°C), humidity and pH (pH>6), acetone cyanohydrin 197 spontaneously breaks down into acetone and free cyanide that can easily evaporate without any 198 other manipulation (Oluwole, 2015; Tshala-katumbay and Spencer, 2007).

199 However, in difficult situations leading to food shortage, people not only rely more exclusively on cassava as caloric source but they also often shortcut the processing of cassava products (Banea et 200 201 al., 1992b; Essers et al., 1992; Tylleskar et al., 1991). As a result, the obtained cassava products 202 contain high quantities of acetone cyanohydrin and linamarin (Banea et al., 1992b; Teles, 2002). 203 Once ingested, the major part of linamarin contained in this insufficiently processed cassava food 204 is eliminated unchanged in the urine (Carlsson et al., 1999; Mlingi et al., 1995; Teles, 2002). A small amount of linamarin is broken down by glucosidases of intestinal flora to form cyanide 205 206 (Padmaja, 1995; Szabo et al., 2010; Tshala-katumbay and Spencer, 2007). Acetone cyanohydrins 207 obtained from this chemical reaction is rapidly transformed in the alkaline environment of the gut 208 into the highly toxic cyanide (Banea et al., 1992b). Cyanide is known as one of the most powerful 209 poisons for human beings (Teles, 2002): a dose as low as 2 mmol (54 mg) can be fatal for a 70 kg 210 adult (Padmaja, 1995; Szabo et al., 2010; Teles, 2002). Cyanide easily diffuses into tissues where 211 it rapidly combines with metallic ions (such as iron and copper) of the mitochondrial cytochrome oxidase and other metalloenzymes (Bhattacharya and Flora, 2015; Teles, 2002). Resulting stable 212 complexes block the cell respiration chain by inhibiting oxygen use, leading to cell death 213 threatening the consumer's life (Bhattacharya and Flora, 2015; Lavigne et al., 2004; Padmaja, 214 1995). It is worth mentioning that acute fatal cyanide intoxication from cassava food is exceptional 215 in areas of cassava consumption (including konzo areas), most likely due to traditional knowledge 216 217 of its fatal risks (Teles, 2002). This finding raises the question about the required dose of cyanide poisoning for konzo; hence the hypothesis of "sublethal" poisoning evoked at times by some 218 authors (Egekeze and Oehme, 1980). This could be subsequent either to ingestion of lower cyanide 219 220 amounts, to partially effective processing of toxic cassava, or to existing detoxification pathways 221 in vivo.

After its release in the organism, cyanide (in this case, predominantly originating from metabolism of cassava cyanogenic glucosides) is metabolized following one major and a few minor pathways detailed in figure 2. In brief, cyanide is first trapped by methemoglobin in the form of cyanmethemoglobin (Pimenta et al., 2010; Schulz, 1984), but this non-enzymatic pathway is
rapidly saturated. The excess cyanide undergoes enzymatic reactions with thiosulfate (SSO3²⁻) to
produce thiocyanate (SCN-) and inorganic sulfate (Nambisan, 2011; Oluwole, 2015; J TorAgbidye et al., 1999). The rhodanese enzyme being abundantly present in the body (Schulz, 1984),
the main rate-limiting factor for this detoxification pathway is the availability of a sulfane sulfur
compound (thiosulfate) (J Tor-Agbidye et al., 1999).

The conversion of cvanide into thiocvanate represents up to 80 % of the in vivo detoxification 231 mechanism (Egekeze and Oehme, 1980). Thiocyanate is more than 100-fold less toxic than cyanide 232 and is easily and rapidly eliminated from the body mainly via the urine (Pimenta et al., 2010; 233 234 Schulz, 1984). Due to this rapid conversion into thiocyanate, high but sublethal doses of cyanogenic 235 glycosides can be consumed over extended periods without clinical symptoms of intoxication (Egekeze and Oehme, 1980). Thiocyanate is the major cyanide metabolite, even in patients with a 236 237 low protein diet (Oluwole and Oludiran, 2013a). It has a short elimination half-life (approximatively 2.7 days (Pimenta et al., 2010; Schulz, 1984)) and remains stable in urine; thus 238 239 urinary thiocyanate concentration represents a good surrogate measure of daily cyanide poisoning during the few preceding days (Lundquist et al., 1995a; Tshala-katumbay and Spencer, 2007). 240 241 Cheap, but accurately sensitive and specific measuring methods of urinary thiocyanate are effectively used in resource-limited countries (Banea et al., 2013; Haque and Bradbury, 1999; 242 243 Lundquist et al., 1995a; Tshala-katumbay and Spencer, 2007). Thus, urinary thiocyanate remains 244 to date the biomarker of choice for cyanide exposure in konzo areas (Oluwole and Oludiran, 2013a), where smoking (that could affect measurements(Madiyal et al., 2018)) is not common. 245 However, thiocyanate synthesis largely relies on SAA that are mainly provided by dietary protein 246 247 intake_(Brosnan and Brosnan, 2006; Nimni et al., 2007). Since konzo patients are nutritionally 248 compromised due to poor protein intake (including SAA (Banea et al., 1997a; Cliff et al., 1985)), using thiocyanate as an indicator of cyanide exposure is likely to underestimate the level of cyanide 249 250 poisoning from toxic cassava.

Alternatively, in the absence of sufficient sulfane sulfur availability, the cyanide anion can be eliminated by other minor pathways, such as oxidation into cyanate (OCN-) (Kassa et al., 2011; J Tor-Agbidye et al., 1999) or reaction with cystine to form L-cysteine and beta-thiocyanoalanine in order to be transformed into 2-Aminothiazoline-4-Carboxylic Acid (ATC), which is deemed metabolically inert and is eliminated in the urine (Kassa et al., 2011; Lundquist et al., 1995b; Nunn et al., 2011). Up to 15 % of potassium cyanide intraperitoneally administered to rats was found to be eliminated as ATC (Egekeze and Oehme, 1980; Lundquist et al., 1995b). A study has reported that cyanide may also react with glutathione in order to be transformed into 2-aminothiazoline-4oxoaminoethanioc acid (Gyamfi et al., 2019).

260

[Figure 2]

261 **3.4. Risk factors of konzo**

262 3.4.1. High dietary cassava-derived cyanide exposure

263 Implication of improperly processed cassava in the occurrence of konzo was already suspected during the very first outbreaks (Cliff et al., 1985; Ministry of health Mozambique, 1984; Tylleskar 264 et al., 1991). Indeed, cassava products contain up to 20-30 times higher amounts of cyanogenic 265 compounds than the WHO safety threshold (10 ppm) during konzo epidemics (Chabwine et al., 266 2011; Ngudi et al., 2003). In agreement with this observation, all subsequent studies conducted in 267 konzo areas, and especially during outbreaks, demonstrated very high thiocyanate levels in konzo 268 patients (up to 500 µmol/L in serum (Banea et al., 1992a) and 1720 µmol/l in urine (Cardoso et al., 269 2004; Kambale et al., 2017; Mlingi et al., 1991; Okitundu et al., 2014)), suggesting a dietary 270 cyanide poisoning. Furthermore, within areas affected by konzo, patients displayed higher urinary 271 thiocyanate levels compared to healthy individuals and to populations from konzo-free areas or 272 from regions where cassava is not frequently consumed (Cliff et al., 1985; Tylleskär et al., 1992). 273 Thus, in the absence of another cyanide source, it is now widely agreed that the origin of high 274 cyanide exposure in the context of konzo outbreaks is linked to dietary improperly processed 275 cassava products (Howlett et al., 1990). In general, as stated above, these populations are 276 undergoing difficult conditions such as drought and war, that lead to a monotonous diet based 277 278 principally on cassava products (Cliff et al., 1985; Nzwalo and Cliff, 2011). Food deprivation due to these difficult living conditions further pushes these populations to introduce short-cuts in 279 cassava processing, which leads to residual high cyanide concentrations in derived cassava 280 products (Banea et al., 1992b; Cliff et al., 1997). 281

However, there are still a number of unanswered questions regarding etiopathogenic mechanisms 282 and causal factors of konzo. First, whether cyanide exposure occurs during the outbreak or at any 283 284 other time outside the epidemic peak remains unclear. In fact, high thiocyanate levels are found at any time in populations living in konzo areas (Banea et al., 1997a; Cliff et al., 2011; Tylleskär et 285 al., 1992), but appear to be even higher during epidemic peaks (Cliff et al., 1985). Second, except 286 for one study in Bandundu (DRC) where konzo patients displayed high urinary thiocyanate levels 287 compared to controls (Boivin et al., 2017), most data collected from konzo affected regions failed 288 to show a significant difference between the urinary thiocyanate levels in konzo patients and 289

individuals without konzo within the same area (Banea et al., 1997a; Tylleskär et al., 1992).
However, caution should be taken in drawing such conclusion as these results might be biased by
the method used to evaluate cyanide exposure, which is based on thiocyanate, of which synthesis
requires sulfur-containing amino-acids that konzo patients are likely deficient for (see below).

294 3.4.2. Protein malnutrition

295 Next to cyanide exposure from incompletely processed cassava processing products, malnutrition 296 is the second important risk factor identified for konzo (Banea et al., 2016; Chabwine et al., 2011). 297 One study conducted in Bandundu (DRC) found indeed that stunted children had significantly 298 higher odds of being affected by konzo, compared to non-stunted children, with an odds ratio of 6.1 (95% CI: 2.80–13.25) (Bumoko et al., 2015). Thus, malnutrition has constantly been mentioned 299 300 as a risk factor of konzo (Banea et al., 2016; Chabwine et al., 2011). As previously mentioned, the main cyanide detoxification pathway requires the presence of thiosulphate, mainly provided by 301 dietary SAA (Nambisan, 2011; J Tor-Agbidye et al., 1999). Accordingly, konzo patients have been 302 often described as being deficient in SAA, as indirectly documented by measurements of urinary 303 concentration of inorganic sulphate, a valid and reliable surrogate for plasma concentration of these 304 SAA (Cliff et al., 1985; Cole and Evrovski, 2000). Plasma SAA concentrations have not been 305 specifically measured in konzo patients. However, patients suffering from TAN, another disease 306 related to cyanide intoxication from improper cassava, were found to have very low plasma levels 307 of SAA, with some of them even completely lacking cysteine (Osuntokun et al., 1968). In general 308 people living in konzo areas have been found to have lower excretion of inorganic sulphate 309 310 compared to people from konzo-free areas (Banea et al., 1997a; Cliff et al., 1985), with konzo 311 patients having even lower levels than unaffected subjects (Cliff et al., 1985).

SAA deficiency constitutes a risk factor for konzo and given its role in synthetizing thiocyanate (the cyanide metabolite used to measure cyanide exposure in the context of konzo), the accuracy of this measurement should be questioned as there could be a high risk of underestimation. Thus, konzo patients could actually be more intoxicated than non-affected individuals, despite the similar thiocyanate levels observed in most studies (see above) (Banea et al., 1997a; Tylleskär et al., 1992). Thus, there is a need to find a more accurate method to assess cyanide exposure in exposed people such as konzo patients who are suffering protein malnutrition, particularly SAA deficiency.

320 3.4.3. Towards etiological factors of konzo

Cyanide exposure from a monotonous improperly processed cassava-based diet and protein 321 malnutrition (in particular SAA deficiency) are now acknowledged as risk factors for konzo. 322 However, although patients as well as supposedly healthy subjects seem to display markers of high 323 cyanide exposure in regions witnessing konzo outbreaks(Banea et al., 1997a; Tylleskär et al., 324 1992), association between high dietary cyanide exposure from poorly processed cassava and 325 occurrence of konzo has not been confirmed in several studies (Adamolekun, 2011). Moreover, the 326 influence of each risk factor has not been precisely determined. In addition, konzo is only described 327 in some specific regions of Africa (Figure 3 and Table 1) while the prevalence of malnutrition is 328 high in many developing countries worldwide (Spencer and Palmer, 2012), as well as the 329 330 consumption of cassava (and presumably improper cassava products due to frequent humanitarian 331 disasters in these regions). It thus appears that the presence of both risk factors is not enough to trigger the appearance of konzo, although the epidemiological link is robust (Nzwalo and Cliff, 332 2011; Tylleskar et al., 1991). It is most likely that konzo occurs as a result of the combination of 333 334 these two risk factors with other parameters at the community level, in terms of individual susceptibility(Kashala-Abotnes et al., 2018) and/or possibly from the environment(Oluwole, 335 2015), further explaining why konzo occurs only in geographically well-defined areas and affects 336 337 only a small portion of the population.

338

[Figure 3]

339 3.5. Konzo determinants and etiopathogenic factors

340 3.5.1. The role of dietary cassava-derived cyanide and cyanogenic metabolites

341 Based on a well-documented epidemiological link (Nzwalo and Cliff, 2011; Tylleskar et al., 1991), it is now widely accepted that toxic cassava varieties containing high levels of cyanogenic 342 343 molecules (mainly linamarin) and yielding toxic cyanide concentrations, play a role in the occurrence of konzo. The few studies investigating serum cyanide levels (Tylleskär et al., 1992) or 344 urinary linamarin (Banea et al., 1997a; Cliff et al., 1999, 1997) found high concentrations both in 345 konzo patients and healthy controls in konzo areas. As detailed above, urines (or sometimes blood) 346 347 thiocyanate levels that usually indicate cassava-derived cyanide poisoning, similarly showed high concentrations in the whole population living in konzo-affected areas (Banea et al., 1997a; 348 Tylleskär et al., 1992). Existence of high concentrations of cyanogenic glucosides, cyanide or 349 cyanide metabolites in communities affected by konzo in comparison to people living in konzo-350 free areas led to the hypothesis that cyanide or one of its derivates might be the causal agent of 351

konzo (Adamolekun, 2011). Indeed, linamarin (Kassa et al., 2011; Rivadeneyra-Dominguez et al., 352 353 2016; Rivadeneyra-Domínguez et al., 2013; Sreeja et al., 2003; Umoh et al., 1985), acetone cyanohydrin (Rivadeneyra-Domínguez et al., 2015; Soler-martín et al., 2010), cyanide (Kimani et 354 al., 2014b, 2014a; Maiorka and Go, 2002) and cyanate (Kassa et al., 2011; Kimani et al., 2014b, 355 2014a; Shaw et al., 1974; John Tor-Agbidye et al., 1999) were experimentally tested in animal 356 357 models and could induce neuronal lesions correlated with neurological symptoms. However, none of them could properly mimic the clinical picture of konzo. Nonetheless, interesting observations 358 359 can be made from the existing data.

A large portion of linamarin, the main cyanogenic compound that determines the rate of cassava 360 361 toxicity (see above), once ingested, is mostly eliminated unchanged in the urine (Carlsson et al., 362 1999; Mlingi et al., 1995; Sreeja et al., 2003; Teles, 2002). One study has suggested that 363 unmetabolized linamarin could be transported to the brain neural cells via a glucose transporter and 364 possibly cause direct toxicity to the brain (Sreeja et al., 2003), but this hypothesis has never been verified in vivo. Only a small part of linamarin is transformed into cyanide (Padmaja, 1995; Szabo 365 366 et al., 2010; Tshala-katumbay and Spencer, 2007). Acetone cyanohydrin, on the other hand, is easily broken down into cyanide owing to the warm, humid and alkaline environment of the 367 368 intestines (Banea et al., 1992b). For this reason, it is suggested that symptoms related to acetone 369 cyanohydrin ingestion may be attributed to cyanide release rather than to the direct effect of acetone 370 cyanohydrin (National Research Council (US) Committee on Acute Exposure Guideline Levels; 371 National Research Council (US) Committee on Toxicology, 2009). Cyanide is a very powerful poison which has to be immediately eliminated from the organism to prevent death. Dietary cyanide 372 exposure at a high dose leads to acute poisoning with headache, dizziness, weakness, visual 373 disturbance, diarrhea, vomiting, nausea, and sometimes death, as observed in some parts of the 374 world (Mlingi et al., 1992). Spastic paraparesis, the key clinical feature of konzo, has never been 375 reported in the context of acute cyanide poisoning. But at a sublethal dose, diverse detoxification 376 pathways can be engaged (see Figure 2), transforming cyanide into less toxic metabolites (Egekeze 377 and Oehme, 1980). Overall, it is unlikely that linamarin, acetone cyanohydrin or cyanide itself are 378 the causative agents of konzo. Instead, cyanide metabolites such as cyanate, thiocyanate, or another 379 380 so far unknown and possibly transient metabolite might be involved.

Under normal conditions, cyanate is produced in trace amounts during the cyanide detoxification
process (Kassa et al., 2011). However, in the case of malnutrition (in particular SAA deficiency),

383 experimental animal data show increased serum cyanate production following cyanide exposure (J

Tor-Agbidye et al., 1999), suggesting that the same would happen in nutritionally compromised

humans (especially those lacking SAA) who are chronically exposed to cyanide poisoning, such as 385 386 most people living in konzo areas. On the other hand, cyanate is a protein-carbamylating agent (Alter et al., 1974; Kassa et al., 2011; Kimani et al., 2013) reducing nerve conduction velocity 387 (John Tor-Agbidye et al., 1999). This is probably the mechanism by which experimental 388 administration of cyanate induced hind limb paralysis in rats (Alter et al., 1974; Kassa et al., 2011), 389 as well as spastic quadriplegia in rhesus monkeys (Shaw et al., 1974). Accordingly, sickle-cell 390 anemic patients who received sodium cyanate as treatment, witnessed motor impairments (Peterson 391 392 et al., 1974). Thus, cyanate appears to impair motor function through defective carbamylation, especially in the presence of protein (SAA) deficiency, thereby becoming a candidate potential 393 394 causative agent of konzo (Kassa et al., 2011).

395 Thiocyanate is the most important cyanide metabolite derived from the cyanide detoxification 396 process, even in people with low dietary protein intake (Egekeze and Oehme, 1980; Nambisan, 397 2011; Oluwole and Oludiran, 2013b). Although being up to 100-fold less toxic than cyanide, thiocyanate may be harmful to the nervous system at high doses (German et al., 1949), causing 398 399 symptoms such as hyperreflexia, muscular fatigue, motor aphasia, convulsive twitching and mental disturbances (Burke and Mutnick, 1994; German et al., 1949). One mechanism underlying these 400 401 neuropathological features could be the enhancement of glutamate binding to AMPA (α-amino-3hydroxy-5-méthyl-4-isoxazolepropionique) receptors (Hawkinson and Espitia, 1997; Murphy et 402 403 al., 1987), which in turn would induce cellular depolarization via calcium and/or sodium entry 404 (Wang and Qin, 2010), and trigger the excitotoxicity cascade and neuronal death (Spencer, 1999). If we consider hyperreflexia observed in animal models as being the minimal equivalent of the 405 406 spastic paraparesis syndrome found in konzo patients, it could be hypothesized that thiocyanateinduced excitotoxicity contributes to the pathogeny of konzo through upper motor neuron damage. 407 408 In support of this assumption, beta-N-Oxalylamino-L-alanine (L-BOAA, the toxic agent found in 409 grass pea (Ngudi et al., 2012) and incriminated in neurolathyrism) is a stereospecific agonist of 410 AMPA receptors in rodents and humans (Spencer and Palmer, 2012) and induces excitotoxic upper 411 motor neuronal death, thereby leading to spastic paraparesis (Ross et al., 1989; Spencer, 1999; M. 412 Van Moorhem et al., 2011; Marijke Van Moorhem et al., 2011). These observations further support 413 thiocyanate as a plausible candidate contributing to the pathogeny of konzo.

So, despite lack of experimental and clinical data, cyanate and thiocyanate appear as potential konzo etiological agents. In contrast, linamarin, as well as cyanide and acetone cyanohydrin, do not seem to be involved. In order to further investigate these hypotheses, appropriate experimental and clinical studies should be conducted, having in mind that there might be additional mechanisms 418 as epidemiological studies have failed to show differentiation between konzo patients and non-419 affected people regarding thiocyanate and cyanide levels.

420 3.5.2. Any role for infectious agents in konzo?

Based on its epidemics occurrence, familial clustering (Carton et al., 1986), and on its prominent
symptoms that evoked some known viral infections, konzo was thought to be an infectious disease
for many years (Carton et al., 1986; World Health Organization, 1996).

424 Table 2 summarizes all studies that investigated the potential infectious origin of konzo. HTLV-1, 425 a virus belonging to the oncovirus family of retroviruses, is the most documented pathogen in konzo (Bangham et al., 2015). Like most retroviruses, HTLV-1 is transmitted by sexual contact, 426 breastfeeding or blood transfusions (Gessain and Mahieux, 2012) and induces in about 1% of 427 infected people, a chronic slowly progressive myelopathy called HTLV-I associated myelopathy 428 429 or tropical spastic paraparesis (HAM/TSP) (Bangham et al., 2015; Zaninovic, 1999). HAM/TSP-430 like syndromes have also been reported in patients infected by other retroviruses such as the HTLV-431 2 and the Human Immunodeficiency Virus (HIV-1 and HIV-2) (Casseb et al., 2008; Posada-Vergara et al., 2006; Zaninovic, 1999). However, in contrast with konzo, HAM/TSP displays a 432 433 slow onset and progressive course spanning from years to several decades. Furthermore, in addition to spastic paraparesis, other myelopathic symptoms such as sensory deficits, pain, urinary and 434 435 sexual disturbances are linked with well-defined spinal cord lesions (Bangham et al., 2015). Although the presence of these retroviruses and konzo may coexist in the same regions, there is to 436 437 date no evidence for a potential association between konzo occurrence and retroviral infections, as more than 99 % of konzo patients display either negative tests for those types of infections 438 439 (Tylleskar et al., 1996) and the few existing case-control studies found a similar frequency of positive serologic tests in konzo patients and healthy controls (Banea et al., 1992a; Howlett et al., 440 1990; Tylleskar et al., 1996; Tylleskär et al., 1992). One study in the DRC reported a HIV-positive 441 442 patient fulfilling the WHO diagnostic criteria of konzo (Chabwine et al., 2011). Some studies investigating other infections, like syphilis (Howlett et al., 1990; Tylleskar et al., 1993), hepatitis 443 A, B and C (Tylleskar et al., 1993) and even schistosomiasis (Howlett et al., 1990), found no 444 evidence of their involvement in the occurrence of konzo. Altogether, the current state of 445 446 knowledge reasonably rules out an infectious origin of konzo.

447 3.5.3. Any role for nutritional deficiencies and oxidative stress?

448 As already mentioned above, most studies show similar levels of markers of cyanide exposure 449 between konzo patients and non-diseased individuals within konzo areas. Thus, there could be 450 factors (for example nutritional, metabolic or genetic) that determine individual susceptibility to
451 konzo (Kashala-Abotnes et al., 2018).

It is well established that nutritional status can significantly influence the neurotoxic action of some chemical agents, or even may be a prerequisite for neurotoxicity to develop, as discussed earlier regarding protein (SAA) deficiency (Spencer and Palmer, 2012). Additionally, oligo-elements deficiency including vitamins might also be involved in the disease occurrence. A role for heavy metal poisoning is also possible, especially in some konzo areas where mining activity is intensive (Weyns et al., 2016). However, none of these hypotheses has been investigated but deserve further attention as being potentially involved in konzo.

As previously stated, SAA deficiency observed in konzo patients can contribute to oxidative stress. 459 460 Indeed, it has been found that methionine and/or cysteine deficiency could lead to glutathione depletion in the central nervous system (Nunn et al., 2011; Spencer and Palmer, 2012). Glutathione, 461 462 in addition to its prominent antioxidant potency, is involved in xenobiotics detoxication (Spencer and Palmer, 2012; John Tor-Agbidye et al., 1999). It has been reported that glutathione may 463 contribute to cyanide detoxification by reacting with cyanide to form 2-aminothiazoline-4-464 oxoaminoethanioc acid (Gyamfi et al., 2019). Furthermore, glutathione depletion enhances in vitro 465 excitotoxicity in cultured cortical neurons (John Tor-Agbidye et al., 1999). Other antioxidant 466 agents such as selenium have been found to be depleted in konzo patients (Bumoko et al., 2015), 467 further supporting the possible role of oxidative stress in konzo. 468

469 Thiamine (vitamin B1) is an essential oligo-element, i.e. mainly provided through dietary intake (Martel et al., 2020). People living in konzo areas are at risk of thiamine deficiency, as they mainly 470 471 rely on a cassava (a poor source of thiamine)-based diet, with poor animal proteins intakes 472 (Adamolekun, 2010). Accordingly, studies on people living in areas with high konzo prevalence, have confirmed a markedly low intake of vitamin B1, including in the majority of supposedly 473 "healthy" subjects (Barclay et al., 2003). Thiamine acts as a coenzyme for several enzymes 474 involved in energy metabolism, and participating in many biochemical and physiological processes 475 476 (Chauhan et al., 2018). Its deficiency is associated with oxidative stress and neurodegeneration in brain tissue (Chauhan et al., 2018; Liu et al., 2017). At a clinical level, vitamin B1 deficiency 477 478 symptoms overlap with less studied konzo symptoms such as cognitive impairment (Johnson and Fox, 2018; Pourhassan et al., 2019) and visual disturbances (Adamolekun, 2011; Gratton and Lam, 479 480 2014), without evident involvement in spastic paraparesis. A few studies have investigated the 481 levels of other group B vitamins (pyridoxin, folic acid and cyanocobalamin) in konzo (Banea et al., 1992a, 1992b; Tylleskar et al., 1993) and TAN patients (Adamolekun, 2011), and found them to 482

be within normal to high ranges. To our knowledge, no study has investigated the status of vitamin A in konzo patients. However, a study in Nigeria has reported the absence of vitamin A deficiency in women and schoolchildren eating cassava (De Moura et al., 2015). Also, vitamin A deficiency and konzo do not share any common symptoms except for visual disturbance, making vitamin A deficiency unlikely to have a causative role in konzo.

It appears that the toxicity of cyanide metabolites could, at an individual level, be directly or indirectly (through oxidative stress) favored or enhanced by different nutritional deficiencies and exacerbated by the state of oxidative stress (Bumoko et al., 2015). However, their putative role in konzo remains to be confirmed.

492 3.5.4. Geographic clustering and environmental factors are important in the occurrence of 493 konzo.

494 For almost a century, konzo outbreaks and sporadic cases have been reported in less than 10 countries in central, eastern, and southern Africa (Table 1 and Figure 3). Even within these 495 496 countries, konzo is unevenly distributed and remains clustered in some restricted areas. As an 497 illustration, in the DRC, which bears the highest prevalence of konzo, regardless of epidemics, 498 cases have been reported only in two provinces: Bandundu in the West (Boivin et al., 2013; Bumoko et al., 2015; Kambale et al., 2017; Luwa E-Andjafono Daniel Okitundu et al., 2018; Luwa 499 500 E-Andjofono Daniel Okitundu et al., 2018; Okitundu et al., 2014) and South-Kivu in the East (Chabwine et al., 2011). In South-Kivu, which is located more than 1000 km away from Bandundu, 501 502 konzo appeared only in Burhinyi, a small remote village, and in Uvira (~ 100 km from Burhinyi) (Chabwine et al., 2011). 503

There is an overlap between the geographical clustering of konzo and the agroecology of cassava in Africa. Up to the early 1990s, the then known five African rural areas affected by konzo figured on a list of 12 areas of high cassava consumption, where more cassava was grown than predicted (Carter and Jones, 1993; Howlett et al., 1992; Tylleskar, 1994). In addition, six of the seven countries affected by konzo so far (see the map on Figure 3) are among the first 20 major cassava production countries worldwide (FAO, 2019). However, the sole agroecology of cassava cannot fully explain this geographic distribution of konzo.

Environmental conditions may also play a major role in the geographic clustering of konzo cases.
A strong relationship was established between low precipitation and konzo epidemics in DRC,
Mozambique, and Tanzania (Oluwole, 2015). However, persistence of sporadic cases of konzo
outside periods of drought, suggests that additional geo-environmental factors might also be

515 involved in appearance of konzo (Imakumbili et al., 2019), like in TAN. In a study conducted in 516 Nigeria, a significant association was found between the cyanogenic content of cassava cultivars, 517 prevalence of TAN and altitude (Oluwole and Oludiran, 2013b). The characteristics of soils where 518 cassava is grown may also impact the total amount of cyanogenic compounds in cassava roots 519 (Imakumbili et al., 2019). Given the role of cassava-derived cyanide exposure in konzo, these 520 factors are likely to also play a role in konzo, but this assumption should be further explored.

The seasonality of konzo also strongly suggests the influence of the environment in the occurrence 521 of konzo, as most konzo epidemics occur during dry seasons (Banea et al., 2015a, 1992a; Chabwine 522 et al., 2011; Tylleskar et al., 1991). In a survey conducted in an area affected by konzo in Tanzania, 523 524 farmers reported increased bitterness of cassava roots during the dry season (Imakumbili et al., 525 2019). This may result from an increase in the amount of cyanogenic glucosides in cassava roots 526 due to natural water stress conditions favored by the structure of soils and by the seasonal changes 527 (Imakumbili et al., 2019; Santisopasri et al., 2001; Tan, 1995). These observations are also consistent with findings from studies by Ernesto et al. in northern Mozambique (Ernesto et al., 528 529 2002) and by Banea et al. in Bandundu (Banea et al., 1997a), who noted a seasonal distribution of urinary thiocyanate levels in subjects from areas affected by konzo, with higher values during the 530 531 dry season. And that also coincided with peak konzo incidence (Banea et al., 2015b, 1992a; Cliff 532 et al., 1985; Ministry of health Mozambique, 1984).

533 **3.6.** Limitations and concluding remarks

534 Konzo is a toxico-nutritional disease with a selective damage of the upper motoneuron, which 535 affects thousands of patients in seven African countries. Throughout the years, even if its clinical 536 picture is already definitely drawn, its risk factors well established and enough knowledge has been gathered to rule out an infectious origin of the disease, little progress has been made in the 537 understanding and the prevention and management of this disease. Almost one century after its 538 identification, many questions remain unanswered: 1° its distribution in person (preferential 539 involvement of children and childbearing women), place (only specific regions in a few countries), 540 and time (mostly during dry season); 2° the causative agent of the disease, and 3° the pathogenic 541 mechanisms leading to the motoneuron damage. 542

543 With no anatomical lesion identified through the whole central nervous system of konzo patients, 544 it seems that the mechanisms involved in the occurrence of konzo take place at sparse cellular 545 and/or at subcellular levels. This is the case also for many other motor neuron diseases (Cluskey 546 and Ramsden, 2001; Rocha et al., 2005; Shaw, 2005). Like other motor neuron diseases, two

interconnected pathophysiologic phenomena seem to be particularly involved in konzo: oxidative 547 548 stress (Bumoko et al., 2015) and excitotoxicity (Spencer, 1999). But more studies are needed to identify their role in konzo. The lack of an appropriate animal model constitutes an important 549 550 limiting factor in the understanding of the pathogenesis of konzo. Furthermore, because konzo is a 551 rare disease, it is very difficult to conduct a large cohort study which can longitudinally follow a 552 population at risk for konzo and find what really happens at the onset of the disease. Except for one study which evaluated blood cyanide and thiocyanate levels in three patients at disease onset 553 554 (within 90 h) (Tylleskär et al., 1992), all available data on konzo were so far gathered from patients at the sequelae phase of the disease, very often many months or years after the onset of the paresis, 555 556 making the understanding of the disease complicated.

Available observational studies have failed to demonstrate the causal relationship between konzo and cyanide poisoning. However, even if thiocyanate remains currently the gold standard to measure cyanide exposure, it may not be the best marker in konzo areas and may underestimate the level of cyanide poisoning in konzo patients, as a larger amount of cyanide is detoxified via other unusual pathways in the context of protein malnutrition (Kassa et al., 2011; J Tor-Agbidye et al., 1999). Furthermore, the compromised nutritional status of affected patients and the oxidative stress may constitute a condition which enhances the neurotoxicity of cyanide compounds.

Geographic and environmental factors probably constitute other factors of susceptibility to the disease, as shown by the clustering of konzo cases in a few specific regions in central, eastern, and southern Africa. Climatic variations have been demonstrated to play a major role in the outbreaks of konzo. According to available data, other geo-environmental factors such as altitude and soil characteristics may also be involved in the occurrence of the disease (Imakumbili et al., 2019; Oluwole and Oludiran, 2013b). But such factors are still to be further investigated in konzo affected areas.

Finally, it is to notice that all konzo affected regions have gone through different humanitarian 571 572 disasters (war, prolonged insecurity with mass population displacements, drought, famine...) before the occurrence of cases (Chabwine et al., 2011; Ministry of health Mozambique, 1984; 573 Nzwalo and Cliff, 2011). All these situations can lead to changes in dietary habits but they may 574 575 also have a direct biological impact on subjects leaving in concerned areas (Stanke et al., 2013). In fact, these disorders can lead to psycho-emotional distress and anxiety (Stanke et al., 2013) which, 576 577 in turn, can shorten the length of chromosome telomeres (Malouff and Schutte, 2017; Wang et al., 578 2017). The length of telomeres is an indicator of human health and aging (Malouff and Schutte, 2017; Sanders and Newman, 2013) and shorter telomers are associated with an increased risk of 579

neurological and cardiovascular diseases, cancers and even high mortality (Malouff and Schutte, 2017). In addition, many individual factors, such as genes, age or gender, can modulate the susceptibility of telomeres to different physical or psychosocial factors (Malouff and Schutte, 2017). Finally, telomeres are very sensitive to oxidative stress (Ahmed and Lingner, 2018; Sanders and Newman, 2013). Whether changes in telomere length are observed in konzo patients and could in turn be used as markers of the disease progression remain speculative. If confirmed, this could, at least partially, explain the geographic, and maybe the individual, clustering of the disease.

In conclusion, almost a century since the description of konzo, its pathogenesis is still unelucidated. However, it affects yearly thousands of patients in specific poor regions in Africa. Konzo occurrence results in an irreversible handicap in the most active portion of the population. For these communities, konzo is a major public and societal health problem. To date, no specific treatment for konzo patients is available due to a gap in the knowledge on konzo. New insights on its etiology are now arising, and, in that context, more research is needed.

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596 Appendix A. Supplementary data

597 The following is the supplementary data to this article: Detailed data for Figure 3.docx.

598 **REFERENCES**

Adamolekun, B., 2010. Etiology of Konzo, epidemic spastic paraparesis associated with
cyanogenic glycosides in cassava: Role of thiamine deficiency? Bola. J. Neurol. Sci. 296,

601 30–33. https://doi.org/10.1016/j.jns.2010.06.016

- Adamolekun, B., 2011. Neurological disorders associated with cassava diet: A review of putative
 etiological mechanisms. Metab. Brain Dis. 26, 79–85. https://doi.org/10.1007/s11011-0119237-y
- Ahmed, W., Lingner, J., 2018. Impact of oxidative stress on telomere biology. Differentiation 99,
 21–27. https://doi.org/10.1016/j.diff.2017.12.002
- 607 Ali Ekangu, R., Kambale Kikandau, J., Vumbi Lelo, G., Kalala Lunganza, R., Yandju Marie-
- 608 Claire, M., Takaisi Kikuni, P., Okitundu Luwa E-Andjafono, D., Mumba Ngoyi, D., Boivin,
- 509 J.M., Tshala- Katumbay, D., 2015. Analysis of motor pathway involvement in Konzo

- disease. J. Neurol. Sci. 357, e496-e497. https://doi.org/10.1016/j.jns.2015.09.287 610
- 611 Allen, T.J., 2010. Konzo in Angola. Cassava Cyanide Dis. Neurolathyrism Netw. 3-4. https://doi.org/10.1371/journal.pntd.0000487 612
- 613 Alter, B.P., Kan, Y.W., Nathan, D.G., 1974. Toxic Effects of High-Dose Cyanate Administration in Rodents. Blood 43, 69-77. https://doi.org/10.1182/blood-2015-06-651331 614
- 615 Banea, J.P., Bikangi, N., Nahimana, G., Nunga, M., Tylleskar, T., Rosling, H., 1992a. High
- prevalence of konzo associated with a food shortage crisis in the Bandundu region of zaire. 616 Ann. Soc. Belg. Med. Trop. (1920). 72, 295-309. 617
- Banea, J.P., Bradbury, J.H., Mandombi, C., Nahimana, D., Denton, I.C., Foster, M.P., Kuwa, N., 618 619 Tshala Katumbay, D., 2015a. Konzo prevention in six villages in the DRC and the
- dependence of konzo prevalence on cyanide intake and malnutrition. Toxicol. Reports 2, 620

609-616. https://doi.org/10.1016/j.toxrep.2015.03.014 621

- Banea, J.P., Bradbury, J.H., Mandombi, C., Nahimana, D., Denton, I.C., Kuwa, N., Tshala 622
- Katumbay, D., 2013. Control of konzo by detoxification of cassava flour in three villages in 623 the Democratic Republic of Congo. Food Chem. Toxicol. 60, 506-513. 624
- https://doi.org/10.1016/j.fct.2013.08.012 625
- Banea, J.P., Bradbury, J.H., Nahimana, D., Denton, I.C., Foster, M.P., Mekob, N., Kuwa, N., 626
- Bokundabi, G., Foley, W.J., 2016. Health factors associated with persistent konzo in four 627 628 villages in the Democratic Republic of Congo (DRC). African J. Food Sci. 10, 1-6. https://doi.org/10.5897/AJFS2015. 629
- 630 Banea, J.P., Bradbury, J.H., Nahimana, D., Denton, I.C., Kuwa, N., 2015b. Survey of the konzo
- prevalence of village people and their nutrition in Kwilu District, Bandundu Province, DRC. 631 632 African J. Food Sci. 9, 43–50. https://doi.org/10.5897/AJFS2014.1206
- Banea, J.P., Poulter, N.H., Rosling, H., 1992b. Shortcuts in cassava processing and risk of dietary 633 634 cyanide exposure in Zaire. Food Nutr. Bull. 14, 137–143.
- 635 Banea, J.P., Tylleskar, T., Gitebo, N., Matadi, N., Gebre-Medhin, M., Rosling, H., 1997a. Geographical and seasonal association between linamarin and cvanide exposure from
- cassava and the upper motor neurone disease konzo in former Zaire. Trop. Med. Int. Health 637
- 2, 1143–1151. 638

- Banea, J.P., Tylleskär, T., Rosling, H., 1997b. Konzo and Ebola in Bandundu region of Zaire. 639 Lancet 349, 621. https://doi.org/10.1016/S0140-6736(05)61569-3 640
- Bangham, C.R.M., Araujo, A., Yamano, Y., Taylor, G.P., 2015. HTLV-1-associated 641
- myelopathy/tropical spastic paraparesis. Nat. Rev. Dis. Prim. 1. 642
- 643 https://doi.org/10.1038/nrdp.2015.12

- Barclay, D. V., Mauron, J., Blondela, A., Cavadini, C., Verwilghen, A.M., Van Geert, C., Dirren,
- H., 2003. Micronutrient intake and status in rural Democratic Republic of Congo. Nutr. Res.
 23, 659–671. https://doi.org/10.1016/S0271-5317(03)00027-7
- 647 Bhattacharya, R., Flora, S.J.S., 2015. Chapter 23 Cyanide Toxicity and its Treatment, in: Gupta,
- 648 R.C.B.T.-H. of T. of C.W.A. (Second E. (Ed.), Handbook of Toxicology of Chemical
- 649 Warfare Agents (Second Edition). Academic Press, Boston, pp. 301–314.
- 650 https://doi.org/https://doi.org/10.1016/B978-0-12-800159-2.00023-3
- Boivin, M.J., Okitundu, D., Makila-Mabe, B., Sombo, M.T., Mumba, D., Sikorskii, A.,
- Mayambu, B., Tshala-Katumbay, D., 2017. Cognitive and motor performance in Congolese
 children with konzo during 4 years of follow-up: a longitudinal analysis. Lancet Glob. Heal.
 5, e936–e947. https://doi.org/10.1016/S2214-109X(17)30267-X
- Boivin, M.J., Okitundu, D., Makila-Mabe Bumoko, G., Sombo, M.-T., Mumba, D., Tylleskar, T.,
 Page, C.F., Tamfum Muyembe, J.-J., Tshala-Katumbay, D., 2013. Neuropsychological
- effects of konzo: a neuromotor disease associated with poorly processed cassava. Pediatrics
 131, e1231-9. https://doi.org/10.1542/peds.2012-3011
- Bonmarin, I., Nunga, M., Perea, W.A., 2002. Konzo outbreak, in the south-west of the
 Democratic Republic of Congo, 1996. J. Trop. Pediatr. 48, 234–238.
- Brosnan, J.T., Brosnan, M.E., 2006. The Sulfur-Containing Amino Acids: An Overview. J. Nutr.
 136, 1636S-1640S. https://doi.org/10.1093/jn/136.6.1636S
- Bumoko, G.M., Sombo, M.T., Okitundu, L.D., Mumba, D.N., Kazadi, K.T., Tamfum-Muyembe,
 J.J., Lasarev, M.R., Boivin, M.J., Banea, J.P., Tshala-Katumbay, D.D., 2014. Determinants
 of cognitive performance in children relying on cyanogenic cassava as staple food. Metab.
 Brain Dis. 29, 359–366. https://doi.org/10.1007/s11011-014-9492-9
- 667 Bumoko, G.M.M., Sadiki, N.H., Rwatambuga, A., Kayembe, K.P., Okitundu, D.L., Mumba
- 668 Ngoyi, D., Muyembe, J.T.J.T., Banea, J.P., Boivin, M.J., Tshala-katumbay, D., Ngoyi,
- 669 D.M., Muyembe, J.T.J.T., Banea, J.P., Boivin, M.J., Tshala-katumbay, D., 2015. Lower
- 670 serum levels of selenium, copper, and zinc are related to neuromotor impairments in
- 671 children with konzo. J. Neurol. Sci. 349, 149–153. https://doi.org/10.1016/j.jns.2015.01.007
- Burke, T.G., Mutnick, A.H., 1994. Treatment of Cyanide and Thiocyanate Toxicity Secondary to
 Sodium Nitroprusside. J. Pharm. Technol. 10, 207–209.
- 674 Cardoso, A.P., Ernesto, M., Nicala, D., Mirione, E., Chavane, L., N'zwalo, H., Chikumba, S.,
- 675 Cliff, J., Mabota, A.P., Haque, M.R., Bradbury, J.H., 2004. Combination of cassava flour
- 676 cyanide and urinary thiocyanate measurements of school children in Mozambique. Int. J.
- 677 Food Sci. Nutr. 55, 183–190. https://doi.org/10.1080/09637480410001725265

- Carlsson, L., Mlingi, N., Juma, A., Ronquist, G., Rosling, H., 1999. Metabolic fates in humans of
 linamarin in cassava flour ingested as stiff porridge. Food Chem. Toxicol. 37, 307–312.
 https://doi.org/10.1016/S0278-6915(99)00015-0
- Carter, S.E., Jones, P.G., 1993. A model of the distribution of cassava in Africa. Appl. Geogr. 13,
 353–371. https://doi.org/10.1016/0143-6228(93)90037-2
- Carton, H., Kayembe, K., Kabeya, Odio, Billiau, a, Maertens, K., 1986. Epidemic spastic
 paraparesis in Bandundu (Zaire). J. Neurol. Neurosurg. Psychiatry 49, 620–627.
 https://doi.org/10.1136/jnnp.49.6.620
- Casseb, J., de Oliveira, A.C.P., Vergara, M.P.P., Montanheiro, P., Bonasser, F., Meilman
 Ferreira, C., Smid, J., Duarte, A.J. da S., 2008. Presence of tropical spastic
- 688 paraparesis/human T-cell lymphotropic virus type 1-associated myelopathy (TSP/HAM)-
- like among HIV-1-infected patients. J. Med. Virol. 80, 392–398.
- 690 https://doi.org/10.1002/jmv.21111
- Chabwine, J., Masheka, C., Balol'ebwami, Z., Maheshe, B., Balegamire, S., Rutega, B., Wa Lola,
 M., Mutendela, K., Bonnet, M.-J., Shangalume, O., Balegamire, J.M., Nemery, B., 2011.
 Appearance of konzo in South-Kivu, a wartorn area in the Democratic Republic of Congo.
- 694 Food Chem. Toxicol. 49, 644–649. https://doi.org/10.1016/j.fct.2010.07.050
- Chauhan, A., Srivastva, N., Bubber, P., 2018. Thiamine Deficiency Induced Dietary Disparity
 Promotes Oxidative Stress and Neurodegeneration. Indian J. Clin. Biochem. 33, 422–428.
 https://doi.org/10.1007/s12291-017-0690-1
- Ciglenečki, I., Eyema, R., Kabanda, C., Taafo, F., Mekaoui, H., Urbaniak, V., 2011. Konzo
 outbreak among refugees from Central African Republic in Eastern region, Cameroon. Food
 Chem. Toxicol. 49, 579–582. https://doi.org/10.1016/j.fct.2010.05.081
- 701 Cliff, J., 2010. Konzo Count. Cassava Cyanide Dis. Neurolathyrism Netw. News 15, 4.
- Cliff, J., Lundqvist, P., Martensson, J., Rosling, H., Sorbo, B., 1985. Association of high cyanide
 and low sulphur intake in cassava-induced spastic paraparesis. Lancet (London, England) 2,
 1211–1213.
- Cliff, J., Muquingue, H., Nhassico, D., Nzwalo, H., Bradbury, J.H., 2011. Konzo and continuing
 cyanide intoxication from cassava in Mozambique. Food Chem. Toxicol. 49, 631–635.
 https://doi.org/10.1016/j.fct.2010.06.056
- Cliff, J., Nicala, D., Saute, F., Givragy, R., Azambuja, G., Taela, A., Chavane, L., Gani, A., 1999.
 Ankle Clonus and Thiocyanate , Linamarin , and Inorganic Sulphate Excretion in School
- 710 Children in Communities with Konzo, Mozambique. J. Trop. Pediatr. 45, 139–142.
- 711 Cliff, J., Nicala, D., Saute, F., Givragy, R., Azambuja, G., Taela, A., Chavane, L., Howarth, J.,

- 712 1997. Konzo associated with war in Mozambique. Trop. Med. Int. Health 2, 1068–1074.
 713 https://doi.org/10.1046/j.1365-3156.1997.d01-178.x
- Cluskey, S., Ramsden, D.B., 2001. Mechanisms of neurodegeneration in amyotrophic lateral
 sclerosis. J. Clin. Pathol. Mol. Pathol. 54, 386–392. https://doi.org/10.1136/mp.54.6.386
- Cole, D.E., Evrovski, J., 2000. The clinical chemistry of inorganic sulfate. Crit. Rev. Clin. Lab.
 Sci. 37, 299–344. https://doi.org/10.1080/10408360091174231
- De Moura, F.F., Moursi, M., Lubowa, A., Ha, B., Boy, E., Oguntona, B., Sanusi, R.A., MaziyaDixon, B., 2015. Cassava Intake and Vitamin A Status among Women and Preschool
- 720 Children in Akwa-Ibom, Nigeria. PLoS One 10, e0129436.
- 721 https://doi.org/10.1371/journal.pone.0129436
- Diasolua Ngudi, D., 2005. Konzo and cassava toxicity: a study of associated nutritional factors in
 the Popokabaka District, Democratic Republic of Congo. Ph D. thesis. Universiteit Gent,
 Belgium.
- Donaghy, M., 1999. Classification and clinical features of motor neurone diseases and motor
 neuropathies in adults. J. Neurol. 246, 331–333. https://doi.org/10.1007/s004150050358
- Egekeze, J.O., Oehme, F.W., 1980. Cyanides and their toxicity: a literature review. Tijdschr.
 Diergeneeskd. 105. https://doi.org/10.1080/01652176.1980.9693766
- 729 Ernesto, M., Cardoso, A.P., Nicala, D., Mirione, E., Massaza, F., Cliff, J., Haque, M.R.,
- Bradbury, J.H., 2002. Persistent konzo and cyanogen toxicity from cassava in northern
 Mozambique. Acta Trop. 82, 357–362. https://doi.org/10.1016/S0001-706X(02)00042-6
- Essers, A.J.A., Alsen, P., Rosling, H., 1992. Insufficient processing of cassava induced acute
 intoxications and the paralytic disease konzo in a rural area of mozambique. Ecol. Food
- 734
 Nutr. 27, 17–27. https://doi.org/10.1080/03670244.1992.9991222

 735
 FAO. 2010. FAOSTAT [W/W/W Descrete] LIBL http://www.faostat/second/
- FAO, 2019. FAOSTAT [WWW Document]. URL http://www.fao.org/faostat/en/#data/QC
 (accessed 12.1.20).
- Food and Agriculture Organization, 2013. Save and Grow : Cassava A guide to sustainable
 production intensification. Roma.
- German, W.F., Messinger, E., Herman, M., 1949. Toxicity of thiocyanates used in treatment of
 hypertension. Ann. Intern. Med. 30, 1054–1059. https://doi.org/10.7326/0003-4819-30-51054
- 742 Gessain, A., Mahieux, R., 2012. Tropical spastic paraparesis and HTLV-1 associated
- myelopathy: Clinical, epidemiological, virological and therapeutic aspects. Rev. Neurol.
 (Paris). 168, 257–269. https://doi.org/10.1016/j.neurol.2011.12.006
- 745 Getahun, H., Lambein, F., Vanhoorne, M., Stuyft, P. Van Der, 2002. Pattern and associated

- factors of the neurolathyrism epidemic in Ethiopia 7, 118–124.
- Gratton, S.M., Lam, B.L., 2014. Visual loss and optic nerve head swelling in thiamine deficiency
 without prolonged dietary deficiency. Clin. Ophthalmol. 8, 1021–1024.
 https://doi.org/10.2147/OPTH.S64228
- 750 Gyamfi, O.A., Bortey-Sam, N., Mahon, S.B., Brenner, M., Rockwood, G.A., Logue, B.A., 2019.
- 751 Metabolism of Cyanide by Glutathione To Produce the Novel Cyanide Metabolite 2-
- Aminothiazoline-4-oxoaminoethanoic Acid. Chem. Res. Toxicol. 32, 718–726.
- 753 https://doi.org/10.1021/acs.chemrestox.8b00384
- Haque, M.R., Bradbury, J.H., 1999. Simple method for determination of thiocyanate in urine.
 Clin. Chem. 45, 1459–1464.
- 756 Hawkinson, J.E., Espitia, S.A., 1997. Effects of thiocyanate and AMPA receptor ligands on (S) -
- 5-fluorowillardiine, (S) -AMPA and (R,S) -AMPA binding. Eur. J. Pharmacol. 329, 213–
 221. https://doi.org/10.1016/S0014-2999(97)89182-0
- Howlett, W.P., Brubaker, G., Mlingi, N., Rosling, H., 1992. A geographical cluster of konzo in
 Tanzania. J. Trop. Geogr. Neurol. 2, 102–108.
- Howlett, W.P., Brubaker, G.R., Mlingi, N., Rosling, H., 1990. Konzo, an epidemic upper motor
 neuron disease studied in Tanzania. Brain 113 (Pt 1, 223–235.
- Imakumbili, M.L.E., Semu, E., Semoka, J.M.R., Abass, A., Mkamilo, G., 2019. Farmers'
 perceptions on the causes of cassava root bitterness: A case of konzo-affected mtwara
 region, Tanzania. PLoS One 14, 1–14. https://doi.org/10.1371/journal.pone.0215527
- Johnson, J.M., Fox, V., 2018. Beyond Thiamine: Treatment for Cognitive Impairment in
 Korsakoff's Syndrome. Psychosomatics 59, 311–317.
- 768 https://doi.org/10.1016/j.psym.2018.03.011
- Joint FAO/WHO Food Standards Programme. Codex Alimentarius Commission, 2009. Codex
 Committee On Contaminants In Foods. Third Session. Discussion Paper On Cyanogenic
 Glycosides.
- 772 Kambale, K.J., Ali, E.R., Sadiki, N.H., Kayembe, K.P., Mvumbi, L.G., Yandju, D.L., Boivin,
- 773 M.J., Boss, G.R., Stadler, D.D., Lambert, W.E., Lasarev, M.R., Okitundu, L.A., Mumba
- 774 Ngoyi, D., Banea, J.P., Tshala-Katumbay, D.D., 2017. Lower sulfurtransferase
- detoxification rates of cyanide in konzo—A tropical spastic paralysis linked to cassava
- cyanogenic poisoning. Neurotoxicology 59, 256–262.
- 777 https://doi.org/10.1016/j.neuro.2016.05.016
- 778 Kashala-Abotnes, E., Okitundu, D., Mumba, D., Boivin, M.J., Tylleskär, T., Tshala-Katumbay,
- D., 2018. Konzo: a distinct neurological disease associated with food (cassava) cyanogenic

- poisoning. Brain Res. Bull. 0–1. https://doi.org/10.1016/j.brainresbull.2018.07.001
- Kasonde, J.M., 2015. Ministerial Statement On Konzo Presented to parliament by the Minister
 of Health.
- 783 Kassa, R.M., Kasensa, N.L., Monterroso, V.H., Kayton, R.J., Klimek, J.E., David, L.L.,
- 784 Lunganza, K.R., Kayembe, K.T., Bentivoglio, M., Juliano, S.L., Tshala-Katumbay, D.D.,
- 785 2011. On the biomarkers and mechanisms of konzo, a distinct upper motor neuron disease
- associated with food (cassava) cyanogenic exposure. Food Chem. Toxicol. 49, 571–578.
 https://doi.org/10.1016/j.fct.2010.05.080
- Kassubek, J., Ludolph, A.C., Muller, H.P., 2012. Neuroimaging of motor neuron diseases. Ther.
 Adv. Neurol. Disord. 5, 119–127. https://doi.org/10.1177/1756285612437562
- 790 Kimani, S., Moterroso, V., Lasarev, M., Kipruto, S., Bukachi, F., Maitai, C., David, L., Tshala-
- 791 Katumbay, D., 2013. Carbamoylation correlates of cyanate neuropathy and cyanide
- poisoning: Relevance to the biomarkers of cassava cyanogenesis and motor system toxicity.
 Springerplus 2, 1–8. https://doi.org/10.1186/2193-1801-2-647
- Kimani, S., Moterroso, V., Morales, P., Wagner, J., Kipruto, S., Bukachi, F., Maitai, C., TshalaKatumbay, D., 2014a. Cross-species and tissue variations in cyanide detoxification rates in
 rodents and non-human primates on protein-restricted diet. Food Chem. Toxicol. 66, 203–
 209. https://doi.org/10.1016/j.fct.2014.01.047
- Kimani, S., Sinei, K., Bukachi, F., Tshala-Katumbay, D., Maitai, C., 2014b. Memory deficits
 associated with sublethal cyanide poisoning relative to cyanate toxicity in rodents. Metab.
 Brain Dis. 29, 105–112. https://doi.org/10.1007/s11011-013-9459-2
- Kimani, S.T., 2011. Neurotoxicity of cassava cyanogens in rodents and non-human primates.
 University of Nairobi.
- Lantum, H., 1998. Spastic paraparesis konzo in the Garoua Boulai Health District, East Province
 Cameroon: a hidden endemic disease. Monograph 85.
- Lavigne, J., Roy, L., Lefebvre, L.F., 2004. Section B 1 : Les cyanures, in: Guide Toxicologique
 Pour Les Urgences En Santé Environnementale. p. 25.
- Liu, D., Ke, Z., Luo, J., 2017. Thiamine Deficiency and Neurodegeneration: the Interplay Among
 Oxidative Stress, Endoplasmic Reticulum Stress, and Autophagy. Mol. Neurobiol. 54,
 5440–5448. https://doi.org/10.1007/s12035-016-0079-9
- Lucasse, C., 1952. Le Kitondji : une paralysie spastique. Ann Soc Belge Med Trop 33, 391–401.
- 811 Lundquist, P., Gedal, B.K., Nilsson, L., 1995a. An Improved Method for Determination of
- Thiocyanate in Plasma and Urine 1). Eur. J. Clin. Chem. Clin. Biochem. 33, 343–349.
- 813 https://doi.org/10.1515/cclm.1995.33.6.343

- Lundquist, P., Kågedal, B., Nilsson, L., Rosling, H., 1995b. Analysis of the cyanide metabolite 2aminothiazoline-4-carboxylic acid in urine by high-performance liquid chromatography.
 Anal. Biochem. https://doi.org/10.1006/abio.1995.1310
- Madiyal, A., Ajila, V., Babu, S.G., Hegde, S., Kumari, S., Madi, M., Achalli, S., Alva, P., Ullal,
 H., 2018. Status of thiocyanate levels in the serum and saliva of non-smokers, ex-smokers
- and smokers. Afr. Health Sci. 18, 727–736. https://doi.org/10.4314/ahs.v18i3.31
- Maiorka, P.C., Go, S.L., 2002. Neuropathologic study of long term cyanide administration to
 goats 40, 1693–1698.
- Makene, W.J., Wilson, J., 1972. Biochemical studies in Tanzanian patients with ataxic tropical
 neuropathy. J. Neurol. Neurosurg. Psychiatry 35, 31–33.
- Malouff, J.M., Schutte, N.S., 2017. A meta-analysis of the relationship between anxiety and

telomere length. Anxiety, Stress Coping 30, 264–272.

- 826 https://doi.org/10.1080/10615806.2016.1261286
- Martel, J.L., Kerndt, C.C., Franklin, D.S., 2020. Vitamin B1 (Thiamine), in: StatPearls. Treasure
 Island (FL).
- Mbelesso, P., Yogo, M.-L., Yangatimbi, E., Paul-Senekian, V. de, Nali, N.M., Preux, P.-M.,
 2009. Outbreak of konzo disease in health region No. 2 of the Central African Republic.

831 Rev. Neurol. (Paris). 165, 466–470. https://doi.org/10.1016/j.neurol.2008.10.020

- Ministry of health Mozambique, 1984. Mantakassa: an epidemic of spastic paraparesis associated
 with chronic cyanide intoxication in a cassava staple area of Mozambique. 1. Epidemiology
 and clinical and laboratory findings in patients. Ministry of Health, Mozambique. Bull.
 World Health Organ. 62, 477–484.
- Mlingi, N., Kimatta, S., Rosling, H., 1991. Konzo, a paralytic disease observed in southern
 Tanzania. Trop Doct 21, 24–25. https://doi.org/10.1177/004947559102100110
- Mlingi, N., Nkya, S., Tatala, S., Rashid, S., Bradbury, H., 2011. Recurrence of konzo in southern
 Tanzania: rehabilitation and prevention using the wetting method. Food Chem. Toxicol. 49,
 673–677. https://doi.org/10.1016/j.fct.2010.09.017
- Mlingi, N., Poulter, N., Rosling, H., 1992. An outbreak of acute intoxications from consumption
 of insufficiently processed cassava in Tanzania. Nutr. Res. 12, 677–687.
- Mlingi, N.L. V, Bainbridge, Z.A., Poulter, N.H., Rosling, H., 1995. Critical stages in cyanogen
 removal during cassava processing in southern Tanzania. Food Chem. 53, 29–33.
- 845 https://doi.org/10.1016/0308-8146(95)95782-2
- 846 Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., 2009. Preferred reporting items for systematic
- reviews and meta- analyses: the PRISMA statement. Ann. Intern. Med. 151, 264–269.

Montagnac, J.A., Davis, C.R., Tanumihardjo, S.A., 2009a. Nutritional value of cassava for use as
a staple food and recent advances for improvement. Compr. Rev. Food Sci. Food Saf. 8,

850 181–194. https://doi.org/10.1111/j.1541-4337.2009.00077.x

Montagnac, J.A., Davis, C.R., Tanumihardjo, S.A., 2009b. Processing techniques to reduce
toxicity and antinutrients of Cassava for use as a staple food. Compr. Rev. Food Sci. Food

853 Saf. 8, 17–27. https://doi.org/10.1111/j.1541-4337.2008.00064.x

- Murphy, D.E., Snowhill, E.W., Williams, M., 1987. Characterization of quisqualate recognition
 sites in rat brain tissue using Dl-[3H]α-amino-3-hydroxy-5-methylisoxazole-4-propionic
- acid (AMPA) and a filtration assay. Neurochem. Res. 12, 775–781.

857 https://doi.org/10.1007/BF00971514

- 858 Mwanza, J.-C., Lysebo, D.E., Kayembe, D.L., Tshala-Katumbay, D., Nyamabo, L.K., Tylleskar,
- T., Plant, G.T., 2003. Visual evoked potentials in konzo, a spastic paraparesis of acute onset
- 860 in Africa. Ophthalmol. J. Int. d'ophtalmologie. Int. J. Ophthalmol. Zeitschrift fur

861 Augenheilkd. 217, 381–386. https://doi.org/73066

- Nambisan, B., 2011. Strategies for elimination of cyanogens from cassava for reducing toxicity
 and improving food safety. Food Chem. Toxicol. 49, 690–693.
- 864 https://doi.org/10.1016/j.fct.2010.10.035
- National Research Council (US) Committee on Acute Exposure Guideline Levels; National
 Research Council (US) Committee on Toxicology, 2009. Acute Exposure Guideline Levels
 for Selected Airborne Chemicals: Volume 7.
- Ngudi, D.D., Kuo, Y.-H., Lambein, F., 2003. Cassava cyanogens and free amino acids in raw and
 cooked leaves. Food Chem. Toxicol. 41, 1193–1197. https://doi.org/10.1016/S02786915(03)00111-X
- Ngudi, D.D., Kuo, Y.-H., Van Montagu, M., Lambein, F., 2012. Research on motor neuron
 diseases konzo and neurolathyrism: trends from 1990 to 2010. PLoS Negl. Trop. Dis. 6,
 e1759. https://doi.org/10.1371/journal.pntd.0001759
- 874 Nhassico, D., Bradbury, J.H., Cliff, J., Majonda, R., Cuambe, C., Denton, I.C., Foster, M.P.,
- 875 Martins, A., Cumbane, A., Sitoe, L., Pedro, J., Muquingue, H., 2016. Use of the wetting
- method on cassava flour in three konzo villages in Mozambique reduces cyanide intake and
 may prevent konzo in future droughts. Food Sci. Nutr. 4, 555–561.
- 878 https://doi.org/10.1002/fsn3.317
- Nimni, M.E., Han, B., Cordoba, F., 2007. Are we getting enough sulfur in our diet? Nutr. Metab.
 (Lond). 4, 24. https://doi.org/10.1186/1743-7075-4-24
- 881 Nunn, P.B., Lyddiard, J.R.A., Christopher Perera, K.P.W., 2011. Brain glutathione as a target for

- aetiological factors in neurolathyrism and konzo. Food Chem. Toxicol. 49, 662–667.
 https://doi.org/10.1016/j.fct.2010.08.037
- Nzwalo, H., Cliff, J., 2011. Konzo: from poverty, cassava, and cyanogen intake to toxiconutritional neurological disease. PLoS Negl. Trop. Dis. 5, e1051.

886 https://doi.org/10.1371/journal.pntd.0001051

- 887 Okitundu, Luwa E-Andjofono Daniel, Ayanne, M.-T.S., Makila-Mabe, G.B., Banea, J.-P.M.,
- Ngoy, D.M., Boivin, M., Tamfum-Muyembe, J.-J., Tshala-Katumbay, D., 2018. Konzo
 global neurological index: a clinical marker of susceptibility and severity of neurocognitive
 deficits in children living in konzo-affected areas. African J. Neurol. Sci. 37, 51–62.
- Okitundu, Luwa E-Andjafono Daniel, Ayanne, M.T.S.S., Makila-Mabe, G.B., Mayambu, J.P.B.,
 Ngoyi, D.M., Boivin, M., Tamfum-Muyembe, J.J., Tshala-Katumbay, D., 2018.
- 893 Socioemotional disorders in children living in Konzo-affected areas, an epidemic paralytic
- disease associated with cyanide poisoning from food in sub-Saharan Africa. Pan Afr. Med.
- J. 31, 1–11. https://doi.org/10.11604/pamj.2018.31.118.11640
- 896 Okitundu, L.E.-A.D., Bumoko, M.-M.G., Sombo, M.T.S.A., Kambale, J.K., Mashukano, N.,
- Kazadi, K.T., Mumba, N.D., Boivin, M.J., Tamfum-Muyembe, J.-J., Banea Mayambu, J.-P.,
 Tshala-Katumbay, D., 2014. Persistence of konzo epidemics in Kahemba, Democratic
 Republic of Congo. phenomenological and socio-economic aspects. Pan Afr. Med. J. 18,
- 900 213. https://doi.org/10.11604/pamj.2014.18.213.4572
- Oluwole, O.S.A., 2015. Cyclical konzo epidemics and climate variability. Ann. Neurol. 77, 371–
 380. https://doi.org/10.1002/ana.24334
- Oluwole, O.S.A., Oludiran, A., 2013a. Normative concentrations of urine thiocyanate in cassava
 eating communities in Nigeria. Int. J. Food Sci. Nutr. 64, 1036–1041.
- 905 https://doi.org/10.3109/09637486.2013.825697
- Oluwole, O.S.A., Oludiran, A., 2013b. Geospatial association of endemicity of ataxic
 polyneuropathy and highly cyanogenic cassava cultivars. Int. J. Health Geogr. 12, 41.
 https://doi.org/10.1186/1476-072X-12-41
- Osuntokun, B.O., Durowoju, J.E., McFarlane, H., Wilson, J., 1968. Plasma Amino-acids in the
 Nigerian Nutritional Ataxic Neuropathy. Br. Med. J. 3, 647–649.
- 911 https://doi.org/10.1136/bmj.3.5619.647
- Padmaja, G., 1995. Cyanide Detoxification in Cassava for Food and Feed Uses. Crit. Rev. Food
 Sci. Nutr. 35, 299–339. https://doi.org/10.1080/10408399509527703
- 914 Peterson, C.M., Tsairis, P., Onishi, A., Lu, Y.S., Grady, R., 1974. Sodium cyanate induced
- polyneuropathy in patients with sickle-cell disease. Ann. Intern. Med. 81, 152–158.

- 916 https://doi.org/10.7326/0003-4819-81-2-152
- Pimenta, E., Calhoun, D.A., Oparil, S., 2010. Hypertensive Emergencies, Second Edi. ed, Cardiac
 Intensive Care. Elsevier Inc. https://doi.org/10.1016/B978-1-4160-3773-6.10028-X
- 919 Posada-Vergara, M.P., Montanheiro, P., Fukumori, L.M.I., Bonasser, F., Duarte, A.J.D.S.,
- 920 Penalva De Oliveira, A.C., Casseb, J., 2006. Clinical and epidemiological aspects of HTLV-
- 921 II infection in São Paulo, Brazil: Presence of tropical spastic paraparesis/HTLV-associated
- 922 myelopathy (TSP/HAM) simile diagnosis in HIV-1-co-infected subjects. Rev. Inst. Med.
- 923 Trop. Sao Paulo 48, 207–210.
- Pourhassan, M., Angersbach, B., Lueg, G., Klimek, C.N., Wirth, R., 2019. Blood Thiamine Level
 and Cognitive Function in Older Hospitalized Patients. J. Geriatr. Psychiatry Neurol. 32, 90–
 96. https://doi.org/10.1177/0891988718819862
- 927 Rivadeneyra-Domínguez, E., Rodríguez-Landa, J.F., 2020. Preclinical and clinical research on
 928 the toxic and neurological effects of cassava (Manihot esculenta Crantz) consumption.
- 929 Metab. Brain Dis. 35, 65–74. https://doi.org/10.1007/s11011-019-00522-0
- Rivadeneyra-Dominguez, E., Rodriguez-Landa, J.F., Article, O., 2016. Motor impairments
 induced by microinjection of linamarin in the dorsal hippocampus of Wistar rats. Neurologia
 31, 516–522. https://doi.org/10.1016/j.nrl.2014.10.018
- Rivadeneyra-Domínguez, E., Vázquez-Luna, a., Díaz-Sobac, R., Eduardo Briones-Céspedes, E.,
 Rodríguez-Landa, J.F., 2015. Contribution of hippocampal area CA1 to acetone
 cyanohydrin-induced loss of motor coordination in rats. Neurologia 32, 1–6.
- 936 https://doi.org/10.1016/j.nrl.2015.11.010
- Rivadeneyra-Domínguez, E., Vázquez-Luna, A., Rodríguez-Landa, J.F., Díaz-Sobac, R., 2013.
 Neurotoxic effect of linamarin in rats associated with cassava (Manihot esculenta Crantz)
- 939
 consumption. Food Chem. Toxicol. 59, 230–235. https://doi.org/10.1016/j.fct.2013.06.004
- Rocha, J.A., Reis, C., Simões, F., Fonseca, J., Mendes Ribeiro, J., 2005. Diagnostic investigation
 and multidisciplinary management in motor neuron disease. J. Neurol. 252, 1435–1447.
 https://doi.org/10.1007/s00415-005-0007-9
- Rosling, H., Gessain, A., de Thé, G., Ebondo, N., Banea, M., Bikangi, N., Kinjanja, K., Nunga,
 K., 1988. Tropical and epidemic spastic paraparesis are different. Lancet MAY 28, 1222–
- 945 1223.
- Ross, S.M., Roy, D.N., Spencer, P.S., 1989. β-N-Oxalylamino-L-Alanine Action on Glutamate
 Receptors. J. Neurochem. 53, 710–715. https://doi.org/10.1111/j.1471-4159.1989.tb11762.x
- 948 Sanders, J.L., Newman, A.B., 2013. Telomere length in epidemiology: A biomarker of aging,
- 949 age-related disease, both, or neither? Epidemiol. Rev. 35, 112–131.

- 950 https://doi.org/10.1093/epirev/mxs008
- 951 Santisopasri, V., Kurotjanawong, K., Chotineeranat, S., Piyachomkwan, K., Sriroth, K., Oates,
- 952 C.G., 2001. Impact of water stress on yield and quality of cassava starch. Ind. Crops Prod.
- 953 13, 115–129. https://doi.org/https://doi.org/10.1016/S0926-6690(00)00058-3
- Sawalha, K., Gonzalez-Toledo, E., Hussein, O., 2019. Role of Magnetic Resonance Imaging in
- Diagnosis of Motor Neuron Disease: Literature Review and Two Case Illustrations. Perm. J.
 23, 1–7. https://doi.org/10.7812/TPP/18-131
- Schulz, V., 1984. Clinical Pharmacokinetics of Nitroprusside, Cyanide, Thiosulphate and
 Thiocyanate. Clin. Pharmacokinet. 9, 239–251. https://doi.org/10.2165/00003088198409030-00005
- Shaw, C., Papayannopoulou, T., Stamatoyannopoulos, G., 1974. Neuropathology of Cyanate
 Toxicity in Rhesus Monkeys 176, 166–176.
- Shaw, P.J., 2005. Molecular and cellular pathways of neurodegeneration in motor neurone
 disease. J. Neurol. Neurosurg. Psychiatry 76, 1046–1057.
- 964 https://doi.org/10.1136/jnnp.2004.048652
- 965 Siddiqi, O.K., Kapina, M., Kumar, R., Ngomah Moraes, A., Kabwe, P., Mazaba, M.L.,
- 966 Hachaambwa, L., Ng'uni, N.M., Chikoti, P.C., Morel-Espinosa, M., Jarrett, J.M., Baggett,
- 967 H.C., Chizema-Kawesha, E., 2020. Konzo outbreak in the Western Province of Zambia.

968 Neurology 94, E1495–E1501. https://doi.org/10.1212/WNL.000000000000017

- 969 Soler-martín, C., Riera, J., Seoane, A., Cutillas, B., Ambrosio, S., Boadas-vaello, P., Llorens, J.,
- 2010. The targets of acetone cyanohydrin neurotoxicity in the rat are not the ones expected
- in an animal model of konzo ☆. Neurotoxicol. Teratol. 32, 289–294.
- 972 https://doi.org/10.1016/j.ntt.2009.11.001
- Spencer, P.S., 1999. Food toxins, ampa receptors, and motor neuron diseases. Drug Metab. Rev.
 31, 561–587. https://doi.org/10.1081/DMR-100101936
- Spencer, P.S., Palmer, V.S., 2012. Interrelationships of undernutrition and neurotoxicity: Food
 for thought and research attention. Neurotoxicology 33, 605–616.
- 977 https://doi.org/10.1016/j.neuro.2012.02.015
- 978 Spencer, P.S., Roy, D.N., Ludolph, A., Hugon, J., Dwivedi, M.P., Schaumburg, H.H., 1986.
 979 Lathyrism: evidence for role of the neuroexcitatory aminoacid BOAA. Lancet 2, 1066–1067.
 980 https://doi.org/10.1016/s0140-6736(86)90468-x
- 981 Sreeja, V.G., Nagahara, N., Li, Q., Minami, M., 2003. New aspects in pathogenesis of konzo:
- neural cell damage directly caused by linamarin contained in cassava (Manihot esculenta
 Crantz). Br. J. Nutr. 90, 467–472. https://doi.org/10.1079/BJN2003902

- Stanke, C., Kerac, M., Prudhomme, C., Medlock, J., Murray, V., 2013. Health Effects of
 Drought: A Systematic Review of the Evidence. PLoS Curr. 1–36.
- 986 https://doi.org/10.1371/currents.dis.7a2cee9e980f91ad7697b570bcc4b004
- Szabo, E.A., Jansson, E., Miles, D., Hambridge, T., Stanley, G., Baines, J., Brent, P., 2010.
 Responding to Incidents of Low Level Chemical Contamination in Food, First Edit. ed,
 Ensuring Global Food Safety. Elsevier Inc. https://doi.org/10.1016/B978-0-12-374845-
- 990 4.00024-2
- Tan, S.L., 1995. Factors affecting cyanide content in cassava (Manihot esculenta Crantz). J. Trop.
 Agric. Food Sci. 23, 121–131.
- Teles, F.F.F., 2002. Chronic poisoning by hydrogen cyanide in cassava and its prevention in
 Africa and Latin America. Food Nutr. Bull. 23, 407–412.
- 995 Tor-Agbidye, J, Palmer, V.S., Lasarev, M.R., Craig, A.M., Blythe, L.L., Sabri, M.I., Spencer,
- P.S., 1999. Bioactivation of cyanide to cyanate in sulfur amino acid deficiency: relevance to
 neurological disease in humans subsisting on cassava. Toxicol. Sci. 50, 228–235.
- 998 Tor-Agbidye, John, Palmer, V.S., Spencer, P.S., Craig, A.M., Blythe, L.L., Sabri, M.I., 1999.
- Sodium cyanate alters glutathione homeostasis in rodent brain: Relationship to
 neurodegenerative diseases in protein-deficient malnourished populations in Africa. Brain
 Res. 820, 12–19. https://doi.org/10.1016/S0006-8993(98)01343-2
- Tshala-Katumbay, D, Edebol Eeg-Olofsson, K., Kazadi-Kayembe, T., Fällmar, P., Tylleskär, T.,
 Kayembe-Kalula, T., 2002. Abnormalities of somatosensory evoked potentials in konzo--an
 upper motor neuron disorder. Clin. Neurophysiol. 113, 10–15.
- Tshala-Katumbay, Desire, Eeg-Olofsson, K.E., Kazadi-Kayembe, T., Tylleskär, T., Fällmar, P.,
 2002. Analysis of motor pathway involvement in konzo using transcranial electrical and
- 1007 magnetic stimulation. Muscle and Nerve 25, 230–235. https://doi.org/10.1002/mus.10029
- 1008 Tshala-Katumbay, D., Eeg-Olofsson, K.E., Tylleskar, T., Kazadi-Kayembe, T., 2001.
- Impairments, disabilities and handicap pattern in konzo--a non-progressive spastic
 para/tetraparesis of acute onset. Disabil. Rehabil. 23, 731–736.
- 1011 Tshala-Katumbay, D., Lukusa, V.M., Eeg-Olofsson, K.E., 2000. EEG Findings in Konzo: A
- 1012 Spastic Para/Tetraparesis of Acute Onset. Clin. EEG Neurosci. 31, 196–200.
- 1013 https://doi.org/10.1177/155005940003100408
- 1014 Tshala-katumbay, D.D., Ngombe, N.N., Okitundu, D., David, L., Westaway, S.K., Boivin, M.J.,
- 1015 Mumba, N.D., Banea, J., 2016. Cyanide and the human brain : perspectives from a model of 1016 food (cassava) poisoning 1378, 50–57. https://doi.org/10.1111/nyas.13159
- 1017 Tshala-katumbay, D.D., Spencer, P.S., 2007. Toxic disorders of the upper motor neuron system,

- in: Handbook of Clinical Neurology. pp. 361–370.
- 1019 Tylleskar, T., 1994. The Causation of Konzo. Studies on a Paralytic Disease in Africa. Uppsala1020 University.
- 1021 Tylleskar, T., Banea, M., Bikangi, N., Fresco, L., Persson, L.A., Rosling, H., 1991.
- Epidemiological Evidence From Zaire for a Dietary Etiology of Konzo, an Upper MotorNeuron Disease. Bull. World Health Organ. 69, 581–589.
- Tylleskär, T., Banea, M., Bikangi, N., Nahimana, G., Persson, L.Å., Rosling, H., 1995. Dietary
 determinants of a non-progressive spastic paraparesis (konzo): A case-referent study in a
 high incidence area of Zaire. Int. J. Epidemiol. 24, 949–956.
- 1027 https://doi.org/10.1093/ije/24.5.949
- Tylleskar, T., Banea, M., Bottiger, B., Thorstensson, R., Biberfeld, G., Rosling, H., 1996. Konzo,
 an epidemic spastic paraparesis in Africa, is not associated with antibodies to HTLV-I, HIV,
 or HIV gag-encoded proteins. J. Acquir. Immune Defic. Syndr. Hum. Retrovirology 12,
- 1031 317–318.
- Tylleskar, T., Howlett, W.P., Rwiza, H.T., Aquilonius, S.M., Stalberg, E., Linden, B., Mandahl,
 A., Larsen, H.C., Brubaker, G.R., Rosling, H., 1993. Konzo: a distinct disease entity with
 selective upper motor neuron damage. J. Neurol. Neurosurg. Psychiatry 56, 638–643.
- Tylleskar, T., Legue, F.D., Kpizingui, E., 1994. Konzo in the Central African Republic.
 Neurology 44, 959–961.
- Tylleskär, T., Rosling, H., Banea, M., Bikangi, N., Cooke, R.D., Poulter, N.H., 1992. Cassava
 cyanogens and konzo, an upper motoneuron disease found in Africa. Lancet 339, 208–211.
 https://doi.org/10.1016/0140-6736(92)90006-O
- 1040 Umoh, I.B., Ogunkoya, F.O., Maduagwu, E.N., Oke, O.L., 1985. Effect of thiamin status on the
 1041 metabolism of linamarin in rats. Ann. Nutr. Metab. 29, 319–324.
- Van Moorhem, Marijke, Decrock, E., De Vuyst, E., De Bock, M., Wang, N., Lambein, F., Van
 Den Bosch, L., Leybaert, L., 2011. L-β-N-oxalyl-α,β-diaminopropionic acid toxicity in
 motor neurons. Neuroreport 22, 131–135. https://doi.org/10.1097/WNR.0b013e3283433027
- 1045 Van Moorhem, M., Lambein, F., Leybaert, L., 2011. Unraveling the mechanism of β-N-oxalyl-
- 1046 α,β -diaminopropionic acid (β -ODAP) induced excitotoxicity and oxidative stress, relevance 1047 for neurolathyrism prevention. Food Chem. Toxicol. 49, 550–555.
- 1048 https://doi.org/10.1016/j.fct.2010.03.054
- Wang, X., Sundquist, K., Hedelius, A., Palmér, K., Memon, A.A., Sundquist, J., 2017. Leukocyte
 telomere length and depression, anxiety and stress and adjustment disorders in primary
- 1051 health care patients. BMC Psychiatry 17, 1–10. https://doi.org/10.1186/s12888-017-1308-0

- Wang, Y., Qin, Z.H., 2010. Molecular and cellular mechanisms of excitotoxic neuronal death.
 Apoptosis 15, 1382–1402. https://doi.org/10.1007/s10495-010-0481-0
- Weyns, Y., Hoex, L., Matthysen, K., 2016. Analysis of the interactive map of artisanal mining
 areas in eastern DR Congo: 2015 update. Antwerp.
- Woldeamanuel, Y.W., Hassan, A., Zenebe, G., 2012. Neurolathyrism: two Ethiopian case reports
 and review of the literature. J. Neurol. 259, 1263–1268. https://doi.org/10.1007/s00415-0116306-4
- World Health Organization, 2010. First WHO report on neglected tropical diseases: working to
 overcome the global impact of neglected tropical diseases, World Health Organization.
 https://doi.org/10.1177/1757913912449575
- 1062 World Health Organization, 1996. Konzo a distinct type of upper motoneuron disease. Wkly.
- 1063 Epidemiol. Rec. 71, 225–228.
- 1064 Zaninovic, V., 1999. On the etiology of tropical spastic paraparesis and human T-cell
- 1065 lymphotropic virus I Associated myelopathy. Int. J. Infect. Dis. 3, 168–177.

1066

FIGURES CAPTIONS







1076 Figure 2. Cyanide detoxification pathways. During the processing of cassava, its cyanogenic glucosides are hydrolyzed by linamarase into acetone cyanohydrin (a). The latter can spontaneously 1077 break down into cyanide in the small intestine due to the hot (>30°C) and humid environment, with 1078 a pH>6 (b). Once released in the blood stream, cyanide is rapidly trapped by methemoglobin to 1079 form cyanmethemoglobin, a stable non-toxic compound (c). But this rapid pathway is quickly 1080 saturated, a larger amount of cyanide (approximately 80 %) is transformed into thiocyanate, the 1081 major cyanide metabolite, via trans-sulfuration reactions catalyzed by the rhodanese and beta-1082 mercaptopyruvate sulfur transferase (d). This pathway requires the availability of a sulfate donor 1083 (thiosulfate). In absence of sufficient available thiosulfate, cvanide is detoxified via other (minor) 1084 pathways including oxidation of cyanide to cyanate, occurring especially in nutritionally 1085 1086 compromised subjects (e), or its combination with hydroxycobalamin to form cyanhydroxycobalamin(f). Cyanide may also react non-enzymatically with cystine to form 2-1087 aminothiazoline-4-Carboxylic acid (g), or with glutathione to form 2-aminothiazoline-4-1088 oxoaminoethanoic acid (h). 1089



Figure 3. Locations of (sporadic and epidemic) konzo cases. This map was generated by ArcGIS

version 10.3. For more information, see the Supplementary material 1.

Country	Province	District	Years	References		
The Democratic Republic of the Congo	Bandundu	Kwango	1928; 1978; 1981; 1992; 1995	(Banea et al., 1997; Carton et al., 1986; Lucasse, 1952)		
		Kwilu	1974; 1996; 2009	(Banea et al., 2015, 1992; Okitundu et al., 2014; Tylleskar et al., 1991)		
	South-Kivu	Mwenga	2001	(Chabwine et al., 2011)		
Mozambique	Cabo Delgado	Chiure	1981	(Cliff et al., 2011)		
	Nampula	Memba	1981; 2005	(Cliff et al., 2011; Ministry of health Mozambique, 1984; Nhassico et al., 2016)		
		Murrupula	1982	(Essers et al., 1992)		
		Mogincual	1992	(Cliff et al., 2011, 1997; Ernesto et al., 2002; Nhassico et al., 2016)		
	Zambezia	Ile	2005	(Cliff et al., 2011)		
Tanzania	Mara	Tarime	1985	(Howlett et al., 1990; Mlingi et al., 2011)		
	Mtwara	Masasi	1988	(Mlingi et al., 1991, 2011)		
		Mtwara rural	2002	(Mlingi et al., 2011)		
		Newala	2002	(Mlingi et al., 2011)		
	Ruvuma	Mbinga	2001	(Mlingi et al., 2011)		
Central African Republic	Nana-Mambéré	Baboua area	1985	(Tylleskar et al., 1994)		
	Nana-Mambéré	Health Region No. 2	2007	(Mbelesso et al., 2009)		
Cameroon	East-Province	Garoua boulai	1998	(Lantum, 1998)		
		Kadei	2007	(Ciglenečki et al., 2011)		
		Lom-et-djerem	2007	(Ciglenečki et al., 2011)		
Angola	Lunda-Norte	Caungula	2010	(Allen, 2010)		
Zambia	Western Province	Mongu	2013 - 2015	(Kasonde, 2015; Siddiqi 2020)		

Table 2 Summary of investigations for infections performed in konzo patients

District/Area, Province (Country)	Year	Investigated Infections	Nb of tested patients	Results	References	
Murrupula district, Nampula (Mozambique)	1982	HTLV-1	7	All tests were negative.	(Essers et al., 1992)	
Kwango, Bandundu, (D.R.C.*)	1985	HTLV-1 HIV-1	10	All tests (10 serum and 10 CSF samples) were negative.	(Carton et al., 1986; De-The et al., 1989)	
Tarime district, Mara Region (Tanzania)	1985	HTLV-1All HTLV-1 and Syphilis (VDRL) tests were negative.Syphilis39Schistosomiasis3/8 controls).		(Howlett et al., 1990)		
Tarime District Mara Region (Tanzania)	1985	HTLV-1 HIV-1&2	61**	All tests were negative.	(De-The et al., 1989)	
Masi-Manimba, Bandundu (D.R.C.*)	1987	HTLV-1 HIV-1&2	15	100 % negative for HTLV-1 & HIV.	(De-The et al., 1989; Rosling et al., 1988)	
Masi-Manimba, Bandundu (D.R.C.*)	nimba, D.R.C.*) 1990 HLTV-1 HIV-1 & 2 3 All tests were negative.		(Tylleskär et al., 1992)			
Pay-Kongila, Bandundu (D.R.C.*)	1990	HLTV-1 HIV-1 & 2	3	All tests performed on patients' sera were negative for HTLV-1 & HIV (but 3 out of 15 tested konzo-free controls were positive for HTLV).	(Banea et al., 1992)	
Tarime district, Mara Region (Tanzania)	1985 & 1991	HLTV-1 HIV-1 & 2 Syphilis Hepatitis A, B & C	2	All tests (on sera and CSF) were negative.	(Tylleskar et al., 1993)	
Masasi district, Mtwara Region (Tanzania)	1991	HLTV-1 HIV-1 & 2	1	All tests were negative.	(Mlingi et al., 1991)	
Baboua area, Nana-Mambéré (Central African Republic)	1994	HIV-1/2 HTLV-1/2	13	All tests were negative for HTLV-1/2. 10/13 were negative for HIV-1/2. 3/13 presented an unspecific reactivity for HIV-1/2.	(Tylleskar et al., 1994)	
Popokabaka, Bandundu (D.R.C.*)	1996	HIV-1/2 HTLV-1/2	38	All tests were negative.	(Tshala-Katumbay et al., 2001)	
Unspecified village, Bandundu (D.R.C.*)1996HIV-1 HTLV-1100 % of sera were negativ ELISA and none fulfilled HIV-1 Westerr		100 % of sera were negative for HIV-1 antibodies on ELISA and none fulfilled the criteria for a positive HIV-1 Western blot reaction.	(Tylleskar et al., 1996)			
Burhinyi, South-Kivu (D.R.C.*)	2005	HIV-1/2	29	1 positive test [using ELISA (Biorad) and Determine (Abott) kits].	(Chabwine et al., 2011)	
Health-Region N°2 (Central African Republic)	2007	HIV-1/2	81	All tests were negative.	(Mbelesso et al., 2009)	
Kahemba, Bandundu (D.R.C.*)	2011	HIV-1/2 HTLV-1/2	123**	All tests were negative.	(Boivin et al., 2013; Bumoko et al., 2015; Kambale et al., 2017; Okitundu et al., 2014)	

Nb: Number; HIV: Human Immunodeficiency Virus; HTLV: Human T-lymphotropic virus; D.R.C.*: The Democratic Republic of the Congo. From 1971 to 1997, D.R.C. was called Zaïre; ** Including the 39 patients from Howlett et al., 1990

Appendix A. Detailed data for Figure 3

This figure shows all locations were konzo cases (both epidemic and sporadic) have been reported. This information was collected from articles retrieved from the literature search. Following data were recorded from articles reporting konzo cases:

- the country, province(s), district(s), and when available, the health area(s) where the cases were recorded,
- the number of reported cases,
- the year of appearance of konzo (if available) or the year when cases were recorded.

Subsequently, geographic coordinates of corresponding health-areas or districts were extracted from Google maps (<u>https://maps.google.com/</u>).

Collected data (see Table S1 below) was compiled in an excel spreadsheet and saved in CSV format. The CSV file was imported in ArcGIS 10.3, and converted to a shapefile format.

The African administrative boundaries shapefile was downloaded from the GADM database (www.gadm.org), version 2.5, July 2015.

Both shapefiles were used as vector layers in ArcGIS 10.3 to plot konzo locations (years of appearance) on the map.

Country	Province	District	Health area	Years	Latitude	Longitude
Angola	Lunda-Norte	Caungula		2010	-8.4270	18.6306
Cameroon	East-Province	Garoua Boulai		1998	6.0290	13.9842
Cameroon	East-Province	Kadei		2007	4.8675	14.2260
Central African Republic	Nana- Mambéré	Baboua area	Baboua area	1985	5.8033	14.8306
Central African Republic	Nana- Mambéré	Health Region No2		2007	4.7136	16.0544
D.R.C.	Bandundu	Kwango	Kahemba	1928, 1996, 2009	-7.2958	18.9621
D.R.C.	Bandundu	Kwango	Popokabaka	1992, 1995	-5.6338	16.6837
D.R.C.	Bandundu	Kwango		1978	-6.1332	18.0155
D.R.C.	Bandundu	Kwango		1981	-4.8089	17.0429
D.R.C.	Bandundu	Kwilu	Masi- Manimba	1974	-4.7714	17.8989
D.R.C.	South-Kivu	Mwenga	Burhinyi	2001	-3.1805	28.4734
Mozambique	Cabo Delgado	Chiure		1981	-13.3760	39.9589
Mozambique	Nampula	Memba	Cava	1981, 2005	-14.2584	40.0981
Mozambique	Nampula	Mogincual	Mujocojo	1992	-15.2552	39.5702
Mozambique	Nampula	Murrupula		1982	-15.4884	38.6874
Mozambique	Zambezia	Ile		2005	-16.0627	37.4067
Tanzania	Mara	Tarime		1985	-1.3433	34.3629
Tanzania	Mtwara	Masasi		1988	-10.7323	38.8089
Tanzania	Mtwara	Mtwara Rural		2002	-10.3860	39.5704
Tanzania	Ruvuma	Mbinga		2001	-10.9428	34.9836
Zambia	Western Province	Mongu	Lwatembu area	2013-2015	-15.2737	23.1503

Table S1. Geographic coordinates of locations where konzo cases have been reported.

D.R.C.: The Democratic Republic of the Congo