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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. Introduction

Konzo is a neurological disease characterized by a sudden onset of symmetrical, non-progressive and irreversible spastic paraparesis due to selective upper motoneuron damage (World Health Organization, 1996). It mostly affects children from 2 years of age and childbearing women (Chabwine et al., 2011; Siddiqi et al., 2020; Tylleskär et al., 1995), causing gait difficulties. The typical spastic gait rapidly develops within a few hours up to one week (Ministry of health Mozambique, 1984; Tylleskär et al., 1995; World Health Organization, 1996). Konzo is an exclusively African disease involving less than ten countries: the Democratic Republic of the 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; 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., 2011; Lantum, 1998), Angola (Allen, 2010), Central African Republic (Mbelesso et al., 2009; 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 zones at the highest risk. Occurrence of konzo epidemics peaks during drought (mostly between June and September), famine or war (Banea et al., 2015a, 1992a; Chabwine et al., 2011; Tylleskar et al., 1991). In some countries, sporadic cases occur outside epidemic periods (Banea et al., 1997a; Chabwine et al., 2011; Ernesto et al., 2002; Tylleskar et al., 1994).

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 known from the late 1800s (Kashala-Abotnes et al., 2018). The prevalence of konzo is most probably underestimated (report of 6788 cases in 2009 (Nzwalo and Cliff, 2011), while the 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 local health practitioners (Nzwalo and Cliff, 2011). Furthermore, affected communities are poor, live in remote areas, have a low education level, and hold several cultural and religious beliefs regarding konzo. As a result, patients with konzo do not resort to health structures (Chabwine et al., 2011; Nzwalo and Cliff, 2011).

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 processed toxic cassava (Manihot esculenta) (Tylleskar et al., 1991). Cassava is a starchy, tuberous root which constitutes a staple food and a major caloric source for about 800 million people mainly residing in tropical regions (Food and Agriculture Organization, 2013). However, cassava protein 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-based diet (such as in konzo-affected areas) run a high risk of suffering from protein malnutrition (Chabwine et al., 2011). Indeed, protein malnutrition and cassava-derived cyanide poisoning are 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 remain unknown (Kassa et al., 2011; Siddiqi et al., 2020).

In the 1980's until late 1990's, several studies were conducted to identify the etiology of konzo and underlying mechanisms. Subsequently, many hypotheses were proposed, including infections such as the human immunodeficiency virus (HIV) and the human T-lymphotropic virus (HTLV), vitamin deficiencies, toxic agents (cyanide and its metabolites), environmental and genetic factors, 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 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 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.

2. Methods

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).

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.

The articles selection process is summarized in Figure 1.

[Figure 1]

3. Results and discussion

3.1. Clinical presentation of konzo

Konzo presents as a symmetric spastic paraparesis (or a tetraparesis in severely affected patients) 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 (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 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 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 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 clinical diagnostic criteria, using the presence of 4 of the following elements: a visible symmetric

spastic abnormality of gait while walking or running; a history of onset of less than 1 week followed 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 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-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 in a tetrapyramidal syndrome (Kassa et al., 2011; Nzwalo and Cliff, 2011; World Health Organization, 1996).

Although in most cases, symptoms associated with konzo appear suddenly and are limited to spastic 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; 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 complaints and vestibular or sensory symptoms, thereby overlapping with tropical ataxic neuropathy (TAN), another disease associated with consumption of toxic cassava, but with a different geographical distribution (Nigeria, Sierra Leonne, India and Cuba) (Adamolekun, 2011; Oluwole, 2015; Osuntokun et al., 1968), except for one report in Tanzania 50 years ago (Makene and Wilson, 1972). Finally, recent studies have shown an association between konzo and impaired 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), although it is not yet clear whether the underlying mechanisms are related to cassava toxicity or other coexisting factors.

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 reason, spinal cord macroscopic lesions should primarily be excluded because their presence conceptually excludes konzo (Tshala-katumbay and Spencer, 2007; World Health Organization, 1996). This probably also applies to brain lesions affecting motor pathways, but available data on brain structure and function of konzo patients are limited (D Tshala-Katumbay et al., 2002; Desire 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 neurolathyrism, a disease with similar symptoms. (Tshala-katumbay and Spencer, 2007; World Health Organization, 1996). The latter is a toxico-nutritional disease associated with a selective

damage of the upper motor neuron, caused by the prolonged ingestion of grass pea (Lathyrus 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-deprived conditions), some epidemiological and clinical characteristics allow differentiation between them: while konzo mainly occurs in children and childbearing women, neurolathyrism mostly affects male adults (Oluwole, 2015; Spencer et al., 1986). Furthermore, these 2 diseases do not occur in the same geographic areas (Ethiopia, Spain, India, Bangladesh for neurolathyrism (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 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 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 day (World Health Organization, 1996)).

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 malnutrition. However, a careful differential diagnosis has to be made to search for (eventually treatable) central nervous system lesions and other toxico-nutritional diseases related to cassava (TAN) or grass pea consumption (neurolathyrism). Lesion should be understood here as "identifiable by currently available tools or published reports", since cellular or subcellular damages below sensitivity of standard diagnostic techniques cannot be excluded (Kashala-Abotnes et al., 2018; Sreeja et al., 2003). Other differential diagnoses possibly involved in or associated with konzo (e.g., vitamin deficiencies) will be more appropriately discussed in the section 3.5. below.

3.2. The lesion site in konzo

It is now well established that the disease mainly affects the upper motoneuron (Donaghy, 1999). However, the exact site of the neuronal damage is still unknown, as the few structural investigations performed in the central nervous system (two autopsies by Trolli in 1937 (Tshala-katumbay and Spencer, 2007; Tylleskar, 1994) and two magnetic resonance imaging in 1991(Tylleskar et al., 1993)) of konzo patients failed to show any macroscopic lesion. This is 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 is currently mainly based on a correct clinical examination searching for signs of the motor neuron

involvement, and exclusion of other neurological conditions that might mimic motor neuron 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, electrophysiological investigations may be useful to support the diagnosis of MND (Rocha et al., 2005). Electrophysiological measurements (especially sensorimotor and visual evoked potentials) 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; Kashala-Abotnes et al., 2018; Mwanza et al., 2003; D Tshala-Katumbay et al., 2002).

The pathogenic processes underlying MND are not yet fully elucidated (Shaw, 2005). Several mechanisms (such as excitotoxicity, oxidative stress, proteins alterations, or genetic factors) are involved in the neurodegeneration observed in MND (Cluskey and Ramsden, 2001; Rocha et al., 2005; Shaw, 2005). A study by Kassa et al. has reported alterations in the expression of proteins 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 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. These observations suggest that the motor neuron degeneration induced by konzo may result from neuronal damage at cellular or molecular levels.

3.3. Dietary cyanide poisoning and its detoxification pathways

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 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 have a better yield (Cliff et al., 1997; Howlett et al., 1990; Imakumbili et al., 2019; Tylleskär et al., 1995). The bitter taste is due to a high content of cyanogenic glucosides, mainly linamarin (about 95 %) and a small quantity of lotaustralin (a methyl-linamarin species) (Montagnac et al., 2009b). Because of their high cyanogenic compounds content and unpleasant taste, the bitter varieties of cassava have to be adequately processed to ensure safety and acceptable taste before consumption (Padmaja, 1995; Szabo et al., 2010).

Most traditional processing methods used in communities consuming cassava, including water-soaking or heap-fermentation of cassava roots, proved to be efficient (Adamolekun, 2011) in reducing cyanogen rates below the toxic threshold of 10 ppm (= mg of total cyanide per kg of

cassava) established by the Food and Agriculture Organization (FAO) and the WHO (Joint FAO/WHO Food Standards Programme. Codex Alimentarius Commission, 2009). During the processing, root integrity is altered, allowing linamarase, an enzyme contained in the root cell walls, to hydrolyze linamarin into glucose and acetone cyanohydrin (Adamolekun, 2011). In specific conditions of temperature (>30°C), humidity and pH (pH>6), acetone cyanohydrin spontaneously breaks down into acetone and free cyanide that can easily evaporate without any other manipulation (Oluwole, 2015; Tshala-katumbay and Spencer, 2007).

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 al., 1992b; Essers et al., 1992; Tylleskar et al., 1991). As a result, the obtained cassava products contain high quantities of acetone cyanohydrin and linamarin (Banea et al., 1992b; Teles, 2002). Once ingested, the major part of linamarin contained in this insufficiently processed cassava food 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 (Padmaja, 1995; Szabo et al., 2010; Tshala-katumbay and Spencer, 2007). Acetone cyanohydrins obtained from this chemical reaction is rapidly transformed in the alkaline environment of the gut into the highly toxic cyanide (Banea et al., 1992b). Cyanide is known as one of the most powerful poisons for human beings (Teles, 2002): a dose as low as 2 mmol (54 mg) can be fatal for a 70 kg adult (Padmaja, 1995; Szabo et al., 2010; Teles, 2002). Cyanide easily diffuses into tissues where 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 complexes block the cell respiration chain by inhibiting oxygen use, leading to cell death threatening the consumer's life (Bhattacharya and Flora, 2015; Lavigne et al., 2004; Padmaja, 1995). It is worth mentioning that acute fatal cyanide intoxication from cassava food is exceptional in areas of cassava consumption (including konzo areas), most likely due to traditional knowledge 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 authors (Egekeze and Oehme, 1980). This could be subsequent either to ingestion of lower cyanide amounts, to partially effective processing of toxic cassava, or to existing detoxification pathways 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 ($SSO3^{2-}$) to produce thiocyanate (SCN-) and inorganic sulfate (Nambisan, 2011; Oluwole, 2015; J Tor-Agbidye 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 cyanide into thiocyanate represents up to 80 % of the in vivo detoxification mechanism (Egekeze and Oehme, 1980). Thiocyanate is more than 100-fold less toxic than cyanide and is easily and rapidly eliminated from the body mainly via the urine (Pimenta et al., 2010; Schulz, 1984). Due to this rapid conversion into thiocyanate, high but sublethal doses of cyanogenic 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 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 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). 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; Lundquist et al., 1995a; Tshala-katumbay and Spencer, 2007). Thus, urinary thiocyanate remains 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. However, thiocyanate synthesis largely relies on SAA that are mainly provided by dietary protein intake (Brosnan and Brosnan, 2006; Nimni et al., 2007). Since konzo patients are nutritionally 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 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-4-oxoaminoethanioc acid (Gyamfi et al., 2019).

[Figure 2]

3.4. Risk factors of konzo

3.4.1. High dietary cassava-derived cyanide exposure

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 et al., 1991). Indeed, cassava products contain up to 20-30 times higher amounts of cyanogenic compounds than the WHO safety threshold (10 ppm) during konzo epidemics (Chabwine et al., 2011; Ngudi et al., 2003). In agreement with this observation, all subsequent studies conducted in konzo areas, and especially during outbreaks, demonstrated very high thiocyanate levels in konzo patients (up to 500 µmol/L in serum (Banea et al., 1992a) and 1720 µmol/l in urine (Cardoso et al., 2004; Kambale et al., 2017; Mlingi et al., 1991; Okitundu et al., 2014)), suggesting a dietary cyanide poisoning. Furthermore, within areas affected by konzo, patients displayed higher urinary thiocyanate levels compared to healthy individuals and to populations from konzo-free areas or from regions where cassava is not frequently consumed (Cliff et al., 1985; Tylleskär et al., 1992). Thus, in the absence of another cyanide source, it is now widely agreed that the origin of high cyanide exposure in the context of konzo outbreaks is linked to dietary improperly processed cassava products (Howlett et al., 1990). In general, as stated above, these populations are undergoing difficult conditions such as drought and war, that lead to a monotonous diet based 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 cassava processing, which leads to residual high cyanide concentrations in derived cassava products (Banea et al., 1992b; Cliff et al., 1997).

However, there are still a number of unanswered questions regarding etiopathogenic mechanisms and causal factors of konzo. First, whether cyanide exposure occurs during the outbreak or at any 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 al., 1992), but appear to be even higher during epidemic peaks (Cliff et al., 1985). Second, except for one study in Bandundu (DRC) where konzo patients displayed high urinary thiocyanate levels compared to controls (Boivin et al., 2017), most data collected from konzo affected regions failed to show a significant difference between the urinary thiocyanate levels in konzo patients and

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).

3.4.2. Protein malnutrition

Next to cyanide exposure from incompletely processed cassava processing products, malnutrition is the second important risk factor identified for konzo (Banea et al., 2016; Chabwine et al., 2011). One study conducted in Bandundu (DRC) found indeed that stunted children had significantly 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 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 dietary SAA (Nambisan, 2011; J Tor-Agbidye et al., 1999). Accordingly, konzo patients have been often described as being deficient in SAA, as indirectly documented by measurements of urinary concentration of inorganic sulphate, a valid and reliable surrogate for plasma concentration of these SAA (Cliff et al., 1985; Cole and Evrovski, 2000). Plasma SAA concentrations have not been specifically measured in konzo patients. However, patients suffering from TAN, another disease related to cyanide intoxication from improper cassava, were found to have very low plasma levels of SAA, with some of them even completely lacking cysteine (Osuntokun et al., 1968). In general people living in konzo areas have been found to have lower excretion of inorganic sulphate compared to people from konzo-free areas (Banea et al., 1997a; Cliff et al., 1985), with konzo 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.

3.4.3. Towards etiological factors of konzo

Cyanide exposure from a monotonous improperly processed cassava-based diet and protein malnutrition (in particular SAA deficiency) are now acknowledged as risk factors for konzo. However, although patients as well as supposedly healthy subjects seem to display markers of high cyanide exposure in regions witnessing konzo outbreaks(Banea et al., 1997a; Tylleskär et al., 1992), association between high dietary cyanide exposure from poorly processed cassava and occurrence of konzo has not been confirmed in several studies (Adamolekun, 2011). Moreover, the influence of each risk factor has not been precisely determined. In addition, konzo is only described in some specific regions of Africa (Figure 3 and Table 1) while the prevalence of malnutrition is high in many developing countries worldwide (Spencer and Palmer, 2012), as well as the consumption of cassava (and presumably improper cassava products due to frequent humanitarian 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, 2011; Tylleskar et al., 1991). It is most likely that konzo occurs as a result of the combination of 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, 2015), further explaining why konzo occurs only in geographically well-defined areas and affects only a small portion of the population.

[Figure 3]

3.5. Konzo determinants and etiopathogenic factors

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

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 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 urinary linamarin (Banea et al., 1997a; Cliff et al., 1999, 1997) found high concentrations both in konzo patients and healthy controls in konzo areas. As detailed above, urines (or sometimes blood) 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; Tylleskär et al., 1992). Existence of high concentrations of cyanogenic glucosides, cyanide or cyanide metabolites in communities affected by konzo in comparison to people living in konzo-free areas led to the hypothesis that cyanide or one of its derivates might be the causal agent of

konzo (Adamolekun, 2011). Indeed, linamarin (Kassa et al., 2011; Rivadeneyra-Dominguez et al., 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 al., 2014b, 2014a; Maiorka and Go, 2002) and cyanate (Kassa et al., 2011; Kimani et al., 2014b, 2014a; Shaw et al., 1974; John Tor-Agbidye et al., 1999) were experimentally tested in animal 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 can be made from the existing data.

A large portion of linamarin, the main cyanogenic compound that determines the rate of cassava toxicity (see above), once ingested, is mostly eliminated unchanged in the urine (Carlsson et al., 1999; Mlingi et al., 1995; Sreeja et al., 2003; Teles, 2002). One study has suggested that unmetabolized linamarin could be transported to the brain neural cells via a glucose transporter and 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 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 intestines (Banea et al., 1992b). For this reason, it is suggested that symptoms related to acetone cyanohydrin ingestion may be attributed to cyanide release rather than to the direct effect of acetone cyanohydrin (National Research Council (US) Committee on Acute Exposure Guideline Levels; 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 exposure at a high dose leads to acute poisoning with headache, dizziness, weakness, visual disturbance, diarrhea, vomiting, nausea, and sometimes death, as observed in some parts of the world (Mlingi et al., 1992). Spastic paraparesis, the key clinical feature of konzo, has never been reported in the context of acute cyanide poisoning. But at a sublethal dose, diverse detoxification pathways can be engaged (see Figure 2), transforming cyanide into less toxic metabolites (Egekeze and Oehme, 1980). Overall, it is unlikely that linamarin, acetone cyanohydrin or cyanide itself are the causative agents of konzo. Instead, cyanide metabolites such as cyanate, thiocyanate, or another 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), 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 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 (John Tor-Agbidye et al., 1999). This is probably the mechanism by which experimental administration of cyanate induced hind limb paralysis in rats (Alter et al., 1974; Kassa et al., 2011), as well as spastic quadriplegia in rhesus monkeys (Shaw et al., 1974). Accordingly, sickle-cell anemic patients who received sodium cyanate as treatment, witnessed motor impairments (Peterson 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 causative agent of konzo (Kassa et al., 2011).

Thiocyanate is the most important cyanide metabolite derived from the cyanide detoxification process, even in people with low dietary protein intake (Egekeze and Oehme, 1980; Nambisan, 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 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 neuropathological features could be the enhancement of glutamate binding to AMPA (α-amino-3-hydroxy-5-méthyl-4-isoxazolepropionique) receptors (Hawkinson and Espitia, 1997; Murphy et al., 1987), which in turn would induce cellular depolarization via calcium and/or sodium entry (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 spastic paraparesis syndrome found in konzo patients, it could be hypothesized that thiocyanate-induced excitotoxicity contributes to the pathogeny of konzo through upper motor neuron damage. In support of this assumption, beta-N-Oxalylamino-L-alanine (L-BOAA, the toxic agent found in grass pea (Ngudi et al., 2012) and incriminated in neurolathyrism) is a stereospecific agonist of AMPA receptors in rodents and humans (Spencer and Palmer, 2012) and induces excitotoxic upper motor neuronal death, thereby leading to spastic paraparesis (Ross et al., 1989; Spencer, 1999; M. Van Moorhem et al., 2011; Marijke Van Moorhem et al., 2011). These observations further support 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

as epidemiological studies have failed to show differentiation between konzo patients and non-affected people regarding thiocyanate and cyanide levels.

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).

Table 2 summarizes all studies that investigated the potential infectious origin of konzo. HTLV-1, 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, breastfeeding or blood transfusions (Gessain and Mahieux, 2012) and induces in about 1% of infected people, a chronic slowly progressive myelopathy called HTLV-I associated myelopathy or tropical spastic paraparesis (HAM/TSP) (Bangham et al., 2015; Zaninovic, 1999). HAM/TSP-like syndromes have also been reported in patients infected by other retroviruses such as the HTLV-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 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 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 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 (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., 1990; Tylleskar et al., 1996; Tylleskär et al., 1992). One study in the DRC reported a HIV-positive 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 A, B and C (Tylleskar et al., 1993) and even schistosomiasis (Howlett et al., 1990), found no evidence of their involvement in the occurrence of konzo. Altogether, the current state of knowledge reasonably rules out an infectious origin of konzo.

3.5.3. Any role for nutritional deficiencies and oxidative stress?

As already mentioned above, most studies show similar levels of markers of cyanide exposure 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).

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

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

469 Thiamine (vitamin B1) is an essential oligo-element, i.e. mainly provided through dietary intake
470 (Martel et al., 2020). People living in konzo areas are at risk of thiamine deficiency, as they mainly
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,
473 have confirmed a markedly low intake of vitamin B1, including in the majority of supposedly
474 "healthy" subjects (Barclay et al., 2003). Thiamine acts as a coenzyme for several enzymes
475 involved in energy metabolism, and participating in many biochemical and physiological processes
476 (Chauhan et al., 2018). Its deficiency is associated with oxidative stress and neurodegeneration in
477 brain tissue (Chauhan et al., 2018; Liu et al., 2017). At a clinical level, vitamin B1 deficiency
478 symptoms overlap with less studied konzo symptoms such as cognitive impairment (Johnson and
479 Fox, 2018; Pourhassan et al., 2019) and visual disturbances (Adamolekun, 2011; Gratton and Lam,
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.,
482 1992a, 1992b; Tylleskar et al., 1993) and TAN patients (Adamolekun, 2011), and found them to

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.

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

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 countries, konzo is unevenly distributed and remains clustered in some restricted areas. As an illustration, in the DRC, which bears the highest prevalence of konzo, regardless of epidemics, 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 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, konzo appeared only in Burhinyi, a small remote village, and in Uvira (~ 100 km from Burhinyi) (Chabwine et al., 2011).

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

involved in appearance of konzo (Imakumbili et al., 2019), like in TAN. In a study conducted in Nigeria, a significant association was found between the cyanogenic content of cassava cultivars, prevalence of TAN and altitude (Oluwole and Oludiran, 2013b). The characteristics of soils where cassava is grown may also impact the total amount of cyanogenic compounds in cassava roots (Imakumbili et al., 2019). Given the role of cassava-derived cyanide exposure in konzo, these 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 of konzo, as most konzo epidemics occur during dry seasons (Banea et al., 2015a, 1992a; Chabwine et al., 2011; Tylleskar et al., 1991). In a survey conducted in an area affected by konzo in Tanzania, farmers reported increased bitterness of cassava roots during the dry season (Imakumbili et al., 2019). This may result from an increase in the amount of cyanogenic glucosides in cassava roots due to natural water stress conditions favored by the structure of soils and by the seasonal changes (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., 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 dry season. And that also coincided with peak konzo incidence (Banea et al., 2015b, 1992a; Cliff et al., 1985; Ministry of health Mozambique, 1984).

3.6. Limitations and concluding remarks

Konzo is a toxico-nutritional disease with a selective damage of the upper motoneuron, which affects thousands of patients in seven African countries. Throughout the years, even if its clinical 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 understanding and the prevention and management of this disease. Almost one century after its identification, many questions remain unanswered: 1° its distribution in person (preferential involvement of children and childbearing women), place (only specific regions in a few countries), and time (mostly during dry season); 2° the causative agent of the disease, and 3° the pathogenic mechanisms leading to the motoneuron damage.

With no anatomical lesion identified through the whole central nervous system of konzo patients, it seems that the mechanisms involved in the occurrence of konzo take place at sparse cellular and/or at subcellular levels. This is the case also for many other motor neuron diseases (Cluskey 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 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 limiting factor in the understanding of the pathogenesis of konzo. Furthermore, because konzo is a rare disease, it is very difficult to conduct a large cohort study which can longitudinally follow a 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 (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, 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 disasters (war, prolonged insecurity with mass population displacements, drought, famine…) before the occurrence of cases (Chabwine et al., 2011; Ministry of health Mozambique, 1984; Nzwalo and Cliff, 2011). All these situations can lead to changes in dietary habits but they may 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, in turn, can shorten the length of chromosome telomeres (Malouff and Schutte, 2017; Wang et al., 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

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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Appendix A. Supplementary data

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

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FIGURES CAPTIONS

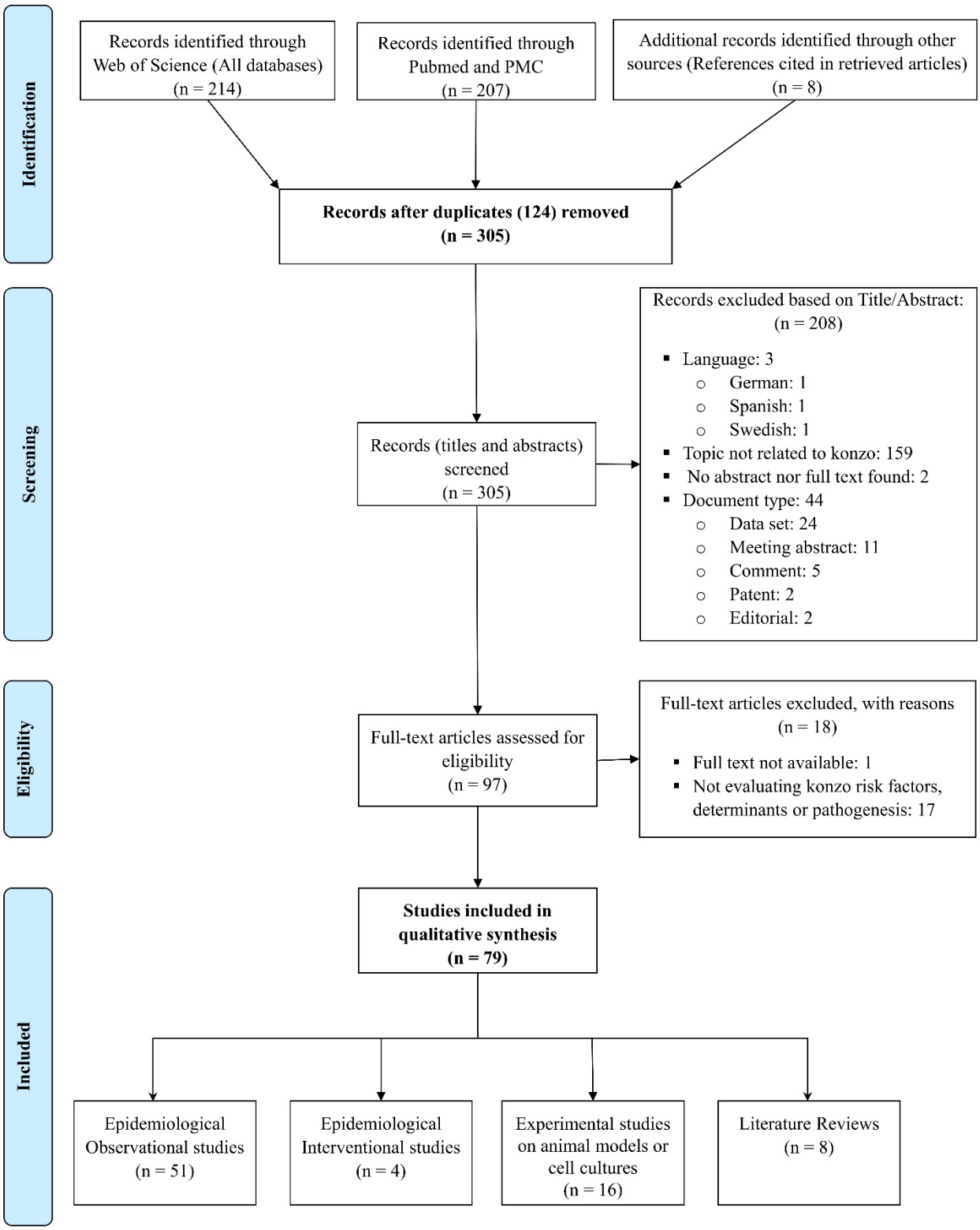

Figure 1. Flowchart summary of the search and selection of the studies.

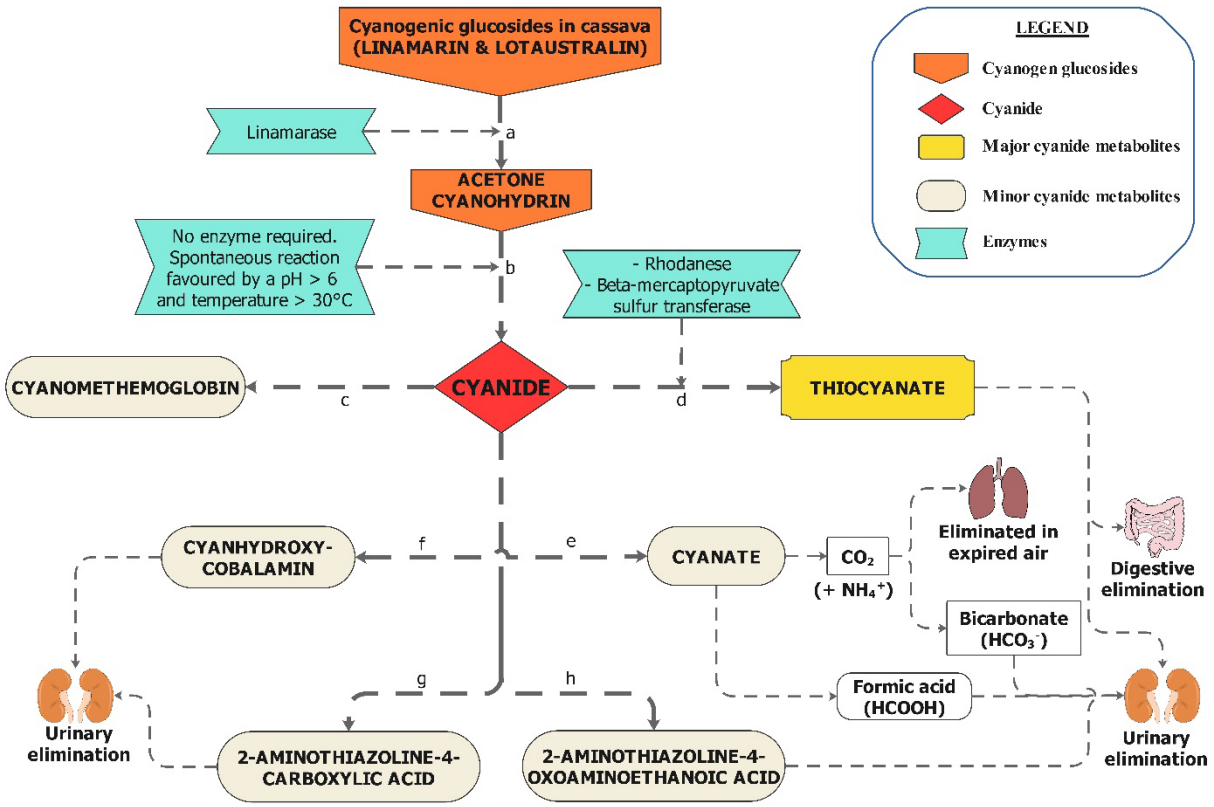

Figure 2. Cyanide detoxification pathways. During the processing of cassava, its cyanogenic glucosides are hydrolyzed by linamarase into acetone cyanohydrin (a). The latter can spontaneously break down into cyanide in the small intestine due to the hot (>30°C) and humid environment, with a pH>6 (b).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

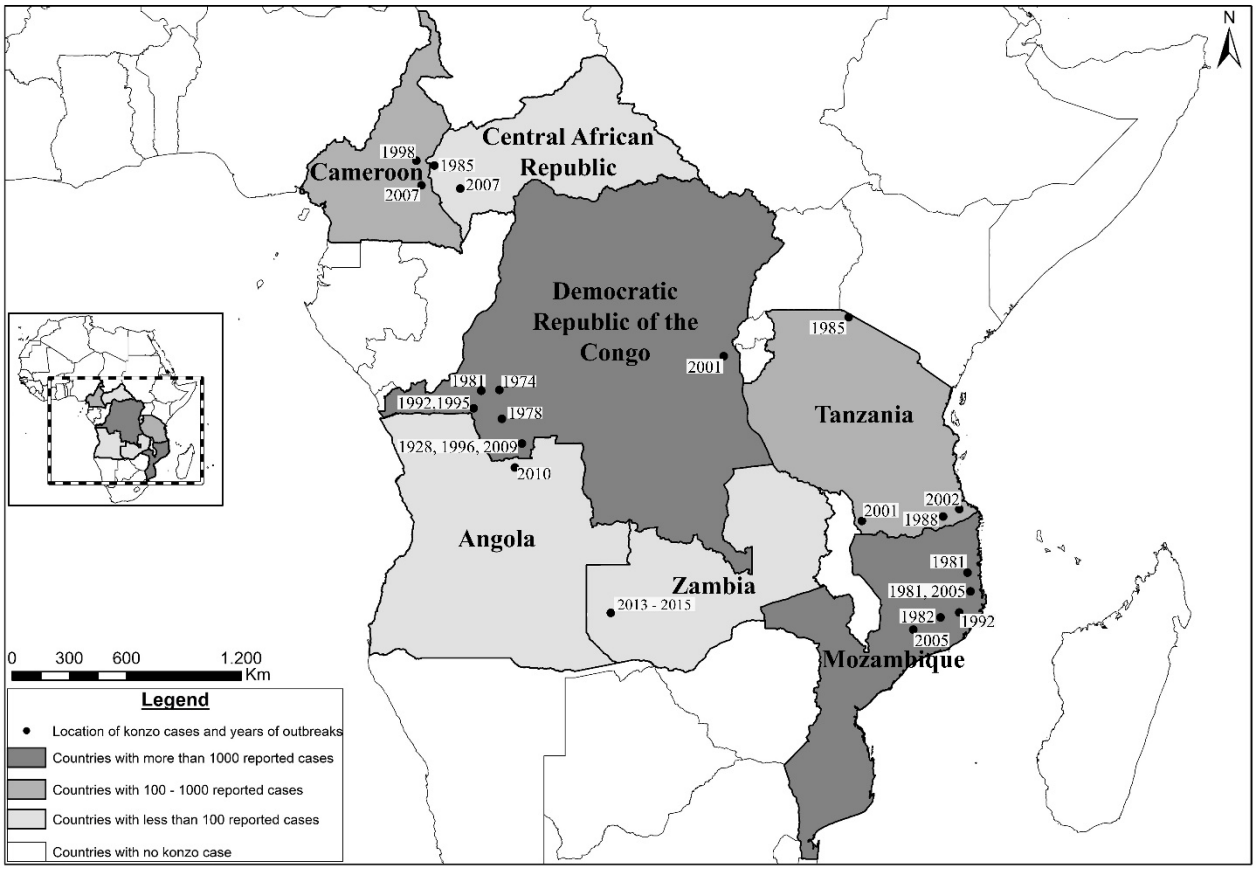

Figure 3. Locations of (sporadic and epidemic) konzo cases. This map was generated by ArcGIS version 10.3. For more information, see the Supplementary material 1.

Table 1. List of locations where konzo outbreaks have been reported

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-1 Syphilis Schistosomiasis	39	All HTLV-1 and Syphilis (VDRL) tests were negative. Schistosoma serology: positive in 8/18 patients (and 3/8 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.*)	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.*)	1996	HIV-1 HTLV-1	33	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 (https://maps.google.com/).

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.

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

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

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