Possible Role of Pandemic AH1N1 Swine Flu Virus in a Childhood Leukemia Cluster

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The causation of acute lymphoblastic leukemia (ALL), the main subtype of childhood cancer, is still unresolved and the difficulty in uncovering a likely multi-factorial etiological pathway is compounded by biological heterogeneity of what is a relatively rare disease.[1, 2]

There is persuasive evidence that the major subset of ALL, B cell precursor or common ALL, is usually initiated pre-natally, *in utero* and that additional secondary genetic changes more proximal to diagnosis are required to trigger overt disease.[2] Any putative etiologic mechanism has to accommodate this pattern of natural history. Among many candidate exposures, infection has long been regarded as a plausible causal agent for childhood ALL,[3, 4] though no specific microbial infections or transforming viruses have been identified.[2, 3] Childhood ALL does not clearly cluster geographically,[3] but this is not surprising if, as postulated, ALL causation involves a rare response to, or outcome of, a common infection of low or modest pathogenicity.[2–4] There are, however, examples of what appear to be significant time/space clusters.[5, 6] Historically, the first of these (in 1957-1960) was in Niles, a suburb of Chicago[5], the most recent (1999-2004), and striking, was in Fallon, Nevada.[6]

We record here a time/space cluster of childhood B cell precursor ALL in Milan, Italy. The seven cases involved had different ages (2-11 years) but were diagnosed within a tight time window of only four weeks (14 December 2009 – 14 January 2010); three cases being associated with a single school and a fourth case being resident in the same area as the school. This led us to suspect that there might be an identifiable trigger or promoting agent for ALL in this particular instance, to which all cases were exposed and which occurred very proximal to the diagnostic time period.

The main biological features of the patients at disease presentation are shown in Supplementary Table 1. All were 'common' B cell precursor (CD10 positive) ALL, five female and two male, with a median age of 7·1 (range 2.1–11.9). Cytogenetic, molecular genetic and genomic alterations were variable but common to those generally present in ALL.[1]

We tested remission DNA of the patients for inherited or constitutive allelic variation in three genes associated with increased risk of B cell precursor ALL: *ARID5B*, *IKZF1* and *CEBPE*.[7] The number of risk alleles present per case varied from one to five (out of six possible; average three) but does not differ from that expected (Supplementary Table 1).

Figure 1 shows the monthly distribution of cases diagnosed with childhood ALL between January 1999 and September 2011 in Milan; the broad-based peak, determined by two consecutive high frequencies observed in December 2009 (three cases) and January 2010 (four cases), has no equivalent in the considered period of 13 years (see also Supplementary Tables 2 and 3).

Figure 2 shows the results of the Scan statistic for the space-time analysis of ALL cases. The Kulldorff's scan method[8] identified only one significant cluster encompassing six administrative areas in the center of Milan within the time interval from 1 December 2009 to 31 January 2010 (*p-value* = 0·017) (see also Supplementary Table 4). The cluster is

composed of four cases, compared to 0·04 expected, corresponding to a rate ratio of 100·0 (95%CI: 26·9-256·0) for the areas and time period identified. The cases are exactly the four children resident in the surroundings of the school, three of them attending the school.

Due to long lasting refurbishment, the primary school was not in operation until September 2009; in the previous year the entire school population was temporarily hosted in the buildings of another school in the same city area. The concentration of all tested physical and chemical agents in the school as well as in the houses of cases did not deviate from normal ranges. Neither parents nor children had noticeable therapeutic exposures to postulated toxicants. None of the parents had professional exposures relevant for leukemia etiology. None of the cases had first level familial malignancies. (Methods and details in Supplementary).

There was an outbreak of pandemic AH1N1 swine flu in Milan between July and September 2009 preceding the ALL diagnosis by around three to six months. Serological screening of plasma antibodies retrieved from blood smears at the time of ALL diagnosis (but before instigation of treatment) revealed that all seven were seropositive (titre range: 160 to 640; see Supplementary Table 5). In plasma samples taken in the same time period from 192 healthy children from Milan, 62 (32.3%) were seropositive suggesting the 100% positivity of the seven cases was significant (p<0.001). Five cases of ALL diagnosed before the outbreak were, as expected, all seronegative. Of four solid pediatric tumors (Wilm's) diagnosed in Milan following the outbreak, only one was seropositive.

If abnormal immune responses to the widespread pandemic of AH1N1 served as a proximal trigger for ALL in the seven cases, then these cases would have to be 'susceptibles'. The 'delayed infection' hypothesis[2] predicts that B cell precursor ALL may be triggered by immune response signals but that risk is contingent upon *deficits* of common infections in infancy that are required to prime the neonatal and infant immune system. This is endorsed by measurement of social contacts in infancy as surrogates for common infections[9–11] and by modelling in mice.[12, 13]

We therefore investigated these characteristics in the seven families involved in the cluster. These were compared to a small series of control cases (Supplementary Table 6). Six out of seven cases were first born compared with nine out of 14 for the controls. All seven cases had parents of medium to high socio-economic class compared with only two of the 20 controls. None of the seven cases attended day care in the first year of life (except for just one week in one case). Two of the seven cases attended day care in the 2-3 years of life compared with 8/14 controls. Of note is the related fact that the school with three cases serves an inner area of Milan of high socio-economic status professional parents.

A plausible explanation for the time/space cluster in Milan is that the AH1N1 epidemic provided a strong immunological or inflammatory signal that triggered conversion to clinical ALL in a small number of individuals harboring clinically silent pre-leukemic clones but who also were at risk of abnormal response to AH1N1 because of a prior deficit of microbial exposures in infancy that prime or modulate the naïve immune system network.[2]

However, the causal explanation for the cluster we propose is based on associations and we cannot formally exclude a role for chance alone. It is therefore tentative. We encourage other investigators to scrutinize incidence data, both nationally and at local levels or within school systems to see if other increases in incidence occurred shortly after the 2009 pandemic of AH1N1.

We do not suggest that AH1N1 has a unique etiological role in childhood ALL or that it poses a public health concern. ALL will, at most, be an extremely rare consequence of AH1N1 infection as part of a multi-factorial etiology. We would anticipate that other rare space/time clusters of childhood ALL may also be due to a promotional effect of infectious agents but not to AH1N1. Influenza viruses may, however, merit further study as indirect, promotional agents for ALL. A prior study noted an association between epidemics of seasonal influenza in the UK and significant increases in the incidence of ALL that follow very shortly (~6 months) thereafter.14 AH1N1, other influenza viruses and, possibly, other viral or bacterial infections may act as triggers or promoters of ALL.

Supplementary information

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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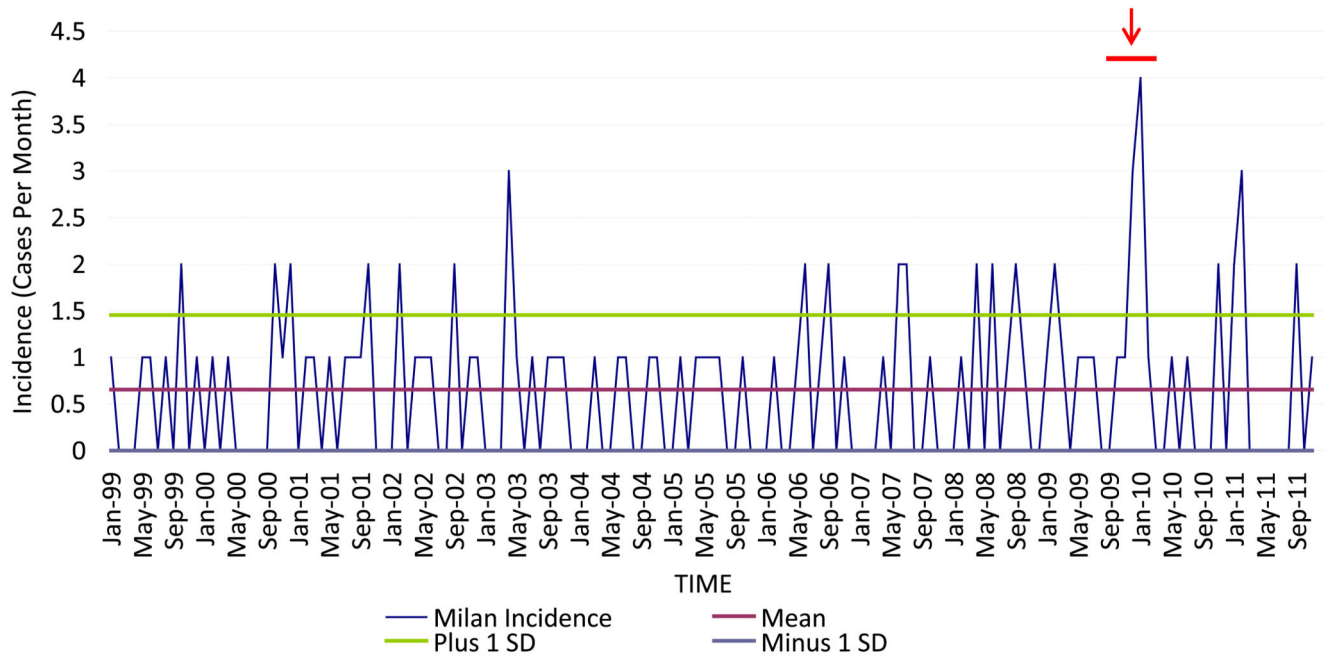
Figure 1.
Monthly distribution of the children diagnosed with ALL between January 1999 and
September 2011 in Milan, Italy. Mean incidence and plus/minus one standard deviation. The
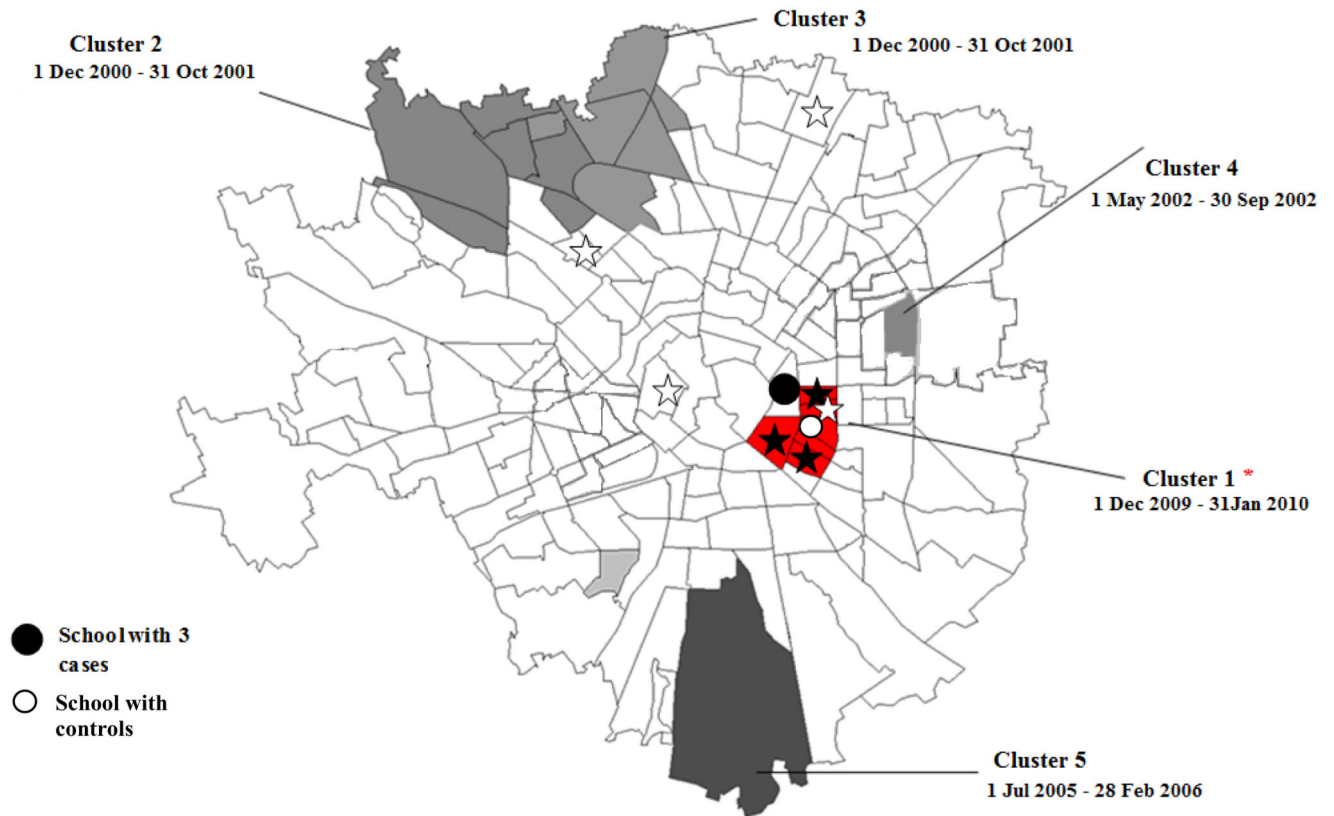arrow indicates the time period in object.

Figure 2.
Representation of most likely spatial ALL clusters identified with Scan statistic in Milan, 1999-2010, considering a monthly time interval. The only statistically significant spatial cluster is in red, while different shades of grey identify non-statistically significant spatial clusters, with specification of corresponding time interval. The site of residence of leukemia cases is indicated by stars (black stars represent the three cases attending the same school). The location of the school of cases is represented by a black dot, while the open dot indicates the school where cases were temporarily hosted and where controls were recruited.