Creatinine related reference ranges for urinary homovanillic acid and vanillylmandelic acid at 6 months of age

M Cole, L Parker, A W Craft, S Bell, G Dale, A C McGill, J A Seviour, J Smith

Abstract

The relationship between homovanillic acid (HVA), vanillylmandelic acid (VMA), and creatinine in the urine of 6 month old babies has been studied and reference ranges in the form of centiles constructed for HVA and VMA against creatinine. Over 10 000 urine samples were collected from babies in four health districts in the north of England. HVA and VMA concentration, either independently or when divided by creatinine concentration, were dependent upon the absolute concentration of creatinine in the sample. After adjustment for creatinine significant differences in the mean concentration of HVA were found between sexes. No such differences were found for VMA. HVA and VMA were also found to be age dependent.

Centiles were constructed using a procedure which makes no distributional assumptions about the data. The net effect of utilising these centiles was to increase the predictive value of a positive screening test from 20% to 40% without any increase in the false negative rate.

(Arch Dis Child 1993; 68: 376–378)

Estimation of the urinary excretion of the catecholamine metabolites homovanillic acid (HVA) and vanillylmandelic acid (VMA) has been a major factor in the diagnosis of neuroblastoma in children since the first report in 1957 of such an association.[1] In over 92% of cases of neuroblastoma the presence of the tumour is accompanied by an increased concentration of urinary HVA and/or VMA excretion.[2] Reference ranges for HVA and VMA have typically been based on small numbers of samples often taken from children hospitalised for unrelated medical conditions. Due to practical difficulties in obtaining timed urine collections and because of the dilution that occurs during the analytical phase it has become customary to express the output of HVA and VMA as their ratio to creatinine (Cr). The statistical methods employed to determine reference ranges for HVA:Cr and VMA:Cr often make distributional assumptions about the data which may not be justifiable. The pilot neuroblastoma studies in the Northern region of England have provided an opportunity to explore the relationship between HVA, VMA, and creatinine.

Methods

Urine samples were collected from 6 month old babies in the four health districts, North Tyneside, South Tyneside, Gateshead, and North

Tees. A detailed audit was undertaken in 1989 in North Tyneside that revealed that 93% of the births for that year had been screened. The samples were obtained, usually by health visitors, by pressing a filter paper onto a wet but unsoiled nappy. Parents were told that they were taking part in a pilot screening programme to try to detect a rare form of cancer. Ethical permission had been obtained from the local ethical committees.

Each sample was subsequently analysed by gas chromatography – mass spectrometry for HVA and VMA and by an automated reaction rate picrate method for creatinine. Full details of urine collection and analytical methods have been described elsewhere.[3 4] Repeat urine samples were collected when there was either an inadequate sample (insufficient urine) or a dilute urine with creatinine concentration of less than 100 µmol/l. The upper limit of normal for HVA:Cr and VMA:Cr had been established during the early phase of the study.[3] The 'cut off' concentration above which babies would be selected for further investigation was chosen as the mean plus 3 SD; 24·2 µmol:mmol creatinine for HVA:Cr and 14·7 µmol:mmol creatinine for VMA:Cr.

This report is based on the results obtained from the first biochemical analysis carried out on each child's urine sample where the creatinine concentration was found to be greater than 100 µmol/l. All children screened between 20 and 40 weeks were included but known cases of neuroblastoma were excluded. Centiles were constructed using a random sample of 1000 eligible results.

The procedure used for centile construction was that of Healy *et al*,[5] which makes no distributional assumptions about the data. The method involves fitting the separate centiles with a series of polynomials whose coefficients are con-
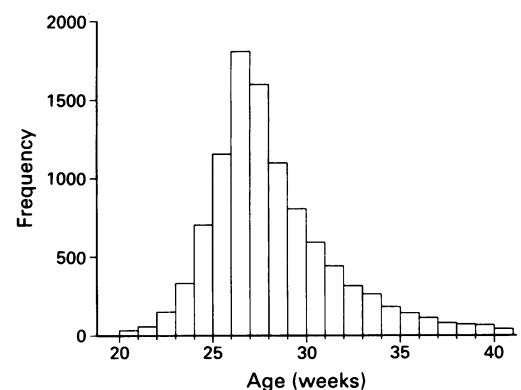
Department of Child
Health, The Medical
School, Framlington
Place, Newcastle upon
Tyne NE2 4HH
M Cole
L Parker
A W Craft
S Bell
G Dale
J Smith

Department of Clinical
Biochemistry, Newcastle
General Hospital
G Dale
A C McGill
J A Seviour
Correspondence to:
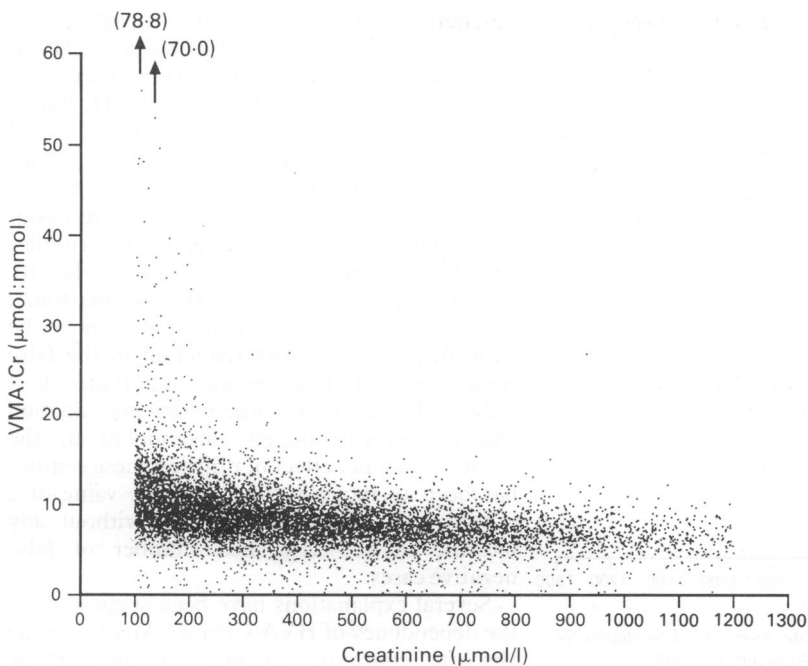Mr Cole.
Accepted 30 September 1992

Figure 1   Histogram of age at screening.

Figure 2   VMA:Cr compared with creatinine (n=10 044).

were dependent upon the absolute concentration of creatinine in the sample. Figure 2 shows the results for VMA:Cr against creatinine. Analysis of covariance (ANCOVA) revealed a significant difference in the mean concentration of HVA between sexes using creatinine as a covariate (p=0·001). This was not the case for VMA (p= 0·662). Figures 3 to 5 show the 97·72th and 99·86th centiles for VMA against creatinine and HVA against creatinine, HVA is shown separately for boys and girls. These centiles are equivalent to the mean plus 2 SD and 3 SD for data which has a Gaussian distribution. Superimposed is a scatter plot of the 10 044 data points from which the random sample was chosen and the centiles were derived.

HVA and VMA are age dependent as well as creatinine dependent. After adjusting for creatinine and sex HVA and VMA were found to be dependent upon age, p=0·003 and p=0·006, respectively (ANCOVA). Although the dependency was highly significant the size of the effect was small. The mean concentration of HVA decreases by 0·076 µmol/l (95% confidence interval (CI) 0·026 to 0·126) per one month increase in age. A change of the same magnitude occurs for a decrease in creatinine of only 8·7 µmol/l. For VMA the mean concentration decreases by 0·039 µmol/l (95% CI 0·011 to 0·067) per one month increase in age. A change in VMA of the same magnitude occurs for a decrease in creatinine of just 6·9 µmol/l.

strained to change smoothly. These were constructed for HVA against creatinine and for VMA against creatinine.

Results

The 10 044 children from whom samples were eligible for this analysis consisted of 5089 boys and 4955 girls. The distribution of age at screening is shown in fig 1. The median age at screening was 27 weeks, range 20 to 40 weeks. There were insufficient data to construct accurate centiles for creatinine concentrations greater than 1200 µmol/l. HVA and VMA concentration, either independently or when divided by creatinine concentration (that is HVA:Cr and VMA:Cr),

Discussion

The introduction of whole population screening programmes for the early detection of neuroblastoma using urinary HVA and VMA has necessitated the establishment of reliable reference ranges for these urinary metabolites. In the present study the distributions of HVA and VMA concentration in the urine, even when divided by creatinine concentration, were found to be dependent upon the absolute concentration of creatinine. Tuchman et al reporting on the results of the first 5000 urine samples from the Quebec study also found that HVA:Cr and VMA:Cr were not independent of the absolute creatinine concentration.[6] However they did not report any difference between males and females.

Problems encountered when dealing with ratios, such as HVA:Cr, have been known for a long time.[7] Recently, Kronmal has again highlighted these problems and proposed modelling the relationship between two variables directly rather than assuming a specific relationship, even implicitly, as with HVA:Cr (RA Kronmal, personal communication). Thompson et al drew a similar conclusion from a study to determine how urinary cotinine concentration should be adjusted for urinary creatinine concentration.[8] The adjustment of HVA and VMA concentration for the absolute concentration of creatinine in this study has been modelled directly.

The procedure usually recommended for the construction of centiles involves calculating the mean and SD of the measurement at each of a series of creatinine concentrations. This is similar to the production of age related centiles,
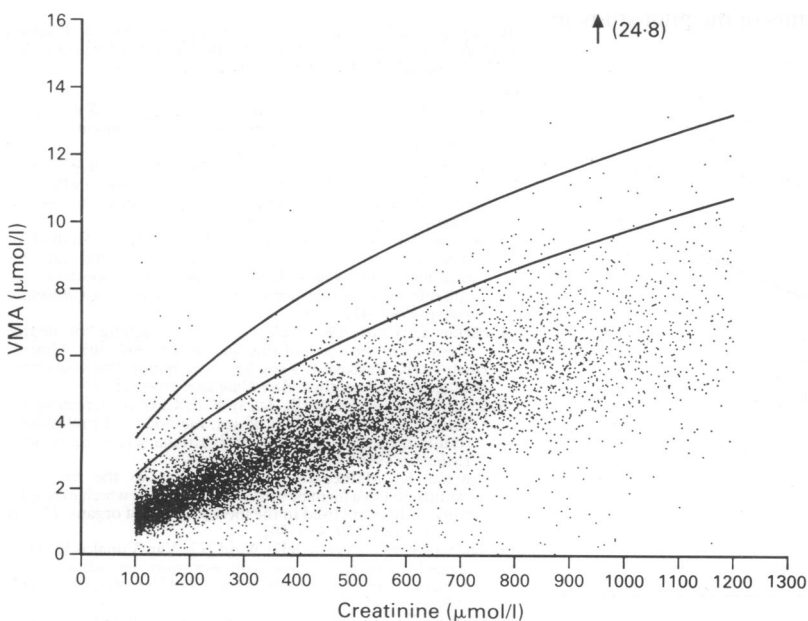

Figure 3   Centiles:VMA compared with creatinine (boys and girls). Centiles shown are those equivalent to 'mean+2 SD and mean+3 SD' for data with a Gaussian distribution (n=10 044).
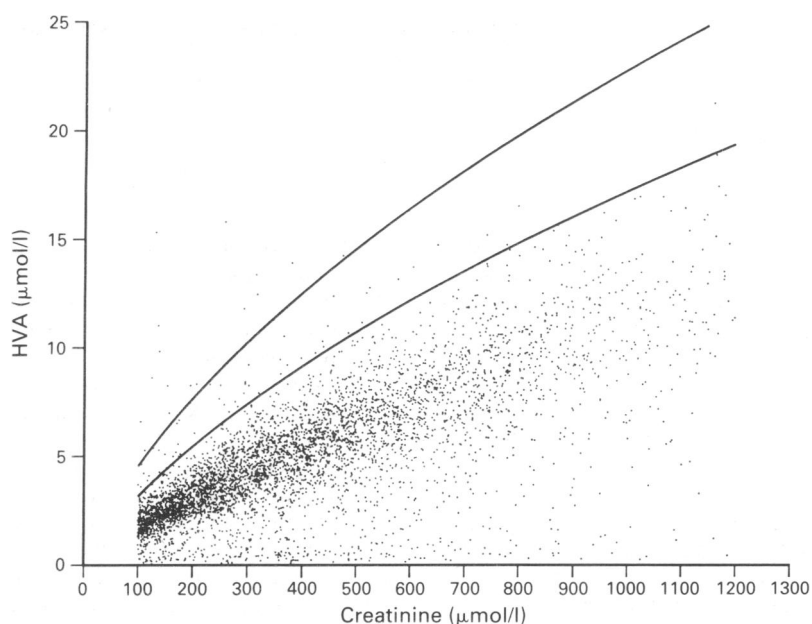
Figure 4   Centiles:HVA compared with creatinine (boys). Centiles shown are those equivalent to 'mean+2 SD and mean+3 SD' for data with a Gaussian distribution (n=5089).

the north of England.[4] Of the 20 829 babies screened 10 were found to have values of HVA: Cr and/or VMA:Cr which were above the original upper limit of mean plus 3 SD, that is 24·2 μmol:mmol creatinine for HVA:Cr and 14·7 μmol:mmol creatinine for VMA:Cr. Two of these 10 children were found to have a neuroblastoma and the other eight were designated false positives. Utilising the centiles reported in this paper only five of the. 10, including the two children with neuroblastoma, would have remained positive (that is above the equivalent centile). Any reduction in the false positive rate is of paramount importance as it reduces the amount of unnecessary investigation and consequent anxiety engendered in the families. The net effect of utilising these centiles has been to increase the predictive value of a positive test from 20% to 40% without any increase in the associated number of false negative cases.

Several explanations have been suggested for the dependency of HVA:Cr and VMA:Cr on the absolute concentration of creatinine. These include the differential extraction of HVA, VMA, and creatinine from the filter paper, the use of wet filter papers – that is drying filter papers at the laboratory rather than at the point of collection, bacterial contamination leading to destruction of creatinine, some effect of creatinine like chromogens, and finally a statistical effect generated by considering the ratio of two random variables. Whatever the explanation the phenomenon exists and must be taken into account when constructing reference ranges for HVA and VMA. Many other urinary biochemical measurements such as albumin, protein and calcium are also traditionally expressed as a ratio to creatinine concentration. The extent to which these ratios are still dependent upon creatinine concentration should be assessed and if required similar methods to those presented in this paper should be adopted for the construction of reference ranges.

for example for height and weight.[9] Centiles are produced by assuming that the distribution of HVA or VMA at a fixed creatinine concentration has a Gaussian distribution. When this assumption is justified this method is highly efficient, however when the assumption is not justified the method is unsatisfactory.[10] In contrast, the procedure of Healy et al used in this paper makes no distributional assumptions.[5]

After adjustment for creatinine, HVA and VMA were found to be age dependent, although the size of the effect was small. It would be possible to produce reference ranges of HVA and VMA against creatinine after adjustment for age, however the adjustment would be of little practical significance.

For 6 month old babies the importance of relating the upper limit of normal for HVA:Cr and VMA:Cr to creatinine concentration is well demonstrated by the results of the pilot study in
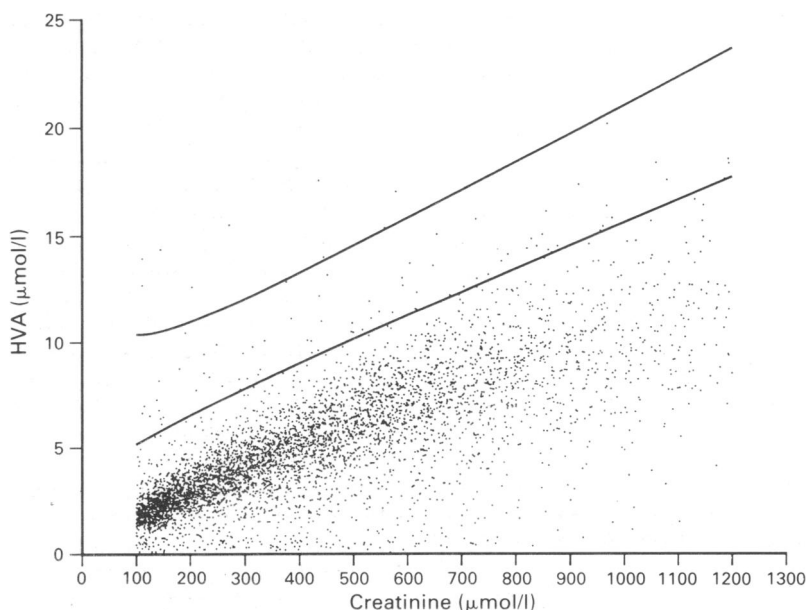
1 Mason GA, Hart-Mercer J, Millar EJ, Strang LB, Wynne NA. Adrenalin-secreting neuroblastoma in an infant. *Lancet* 1957; ii: 322–5.
2 Tuchman M, Ramnaraine ML, Woods WG, Krivit W. Three years experience with random urinary homovanillic and vanillylmandelic acid levels in the diagnosis of neuroblastoma. *Pediatrics* 1987; **79**: 203–5.
3 Seviour JA, McGill AC, Dale G, Craft AW. Method of measurement of urinary homovanillic acid and vanillylmandelic acid by gas chromatography-mass spectrometry suitable for neuroblastoma screening. *Journal of Chromatography* 1988; **432**: 273–7.
4 Parker L, Craft AW, Dale G, et al. Screening for neuroblastoma in the north of England. *BMJ* 1992; **305**: 1260–3.
5 Healy MJR, Rasbash J, Yang M. Distribution-free estimation of age-related centiles. *Ann Hum Biol* 1988; **15**: 17–22.
6 Tuchman M, Lemieux B, Auray-Blais C, et al. Screening for neuroblastoma at 3 weeks of age: methods and preliminary results from the Quebec neuroblastoma screening project. *Pediatrics* 1990; **86**: 765–73.
7 Pearson K. Mathematical contributions to the theory of evolution. On a form of spurious correlation which may arise when indices are used in the measurement of organs. *Proc R Soc Med* 1897; **60**: 489–502.
8 Thompson SG, Barlow RD, Wald NJ, Van Vunakis H. How should urinary cotinine concentration be adjusted for urinary creatinine concentration? *Clin Chim Acta* 1990; **187**: 289–96.
9 Royston P. Constructing time-specific reference ranges. *Stat Med* 1991; **10**: 675–90.
10 Healy MJR. Statistics of growth standards. In Faulkner F, Tanner JM, eds. *Human growth. Vol 1. Principles and prenatal growth.* New York: Plenum Press, 1986: 47–58.

Figure 5   Centiles:HVA compared with creatinine (girls). Centiles shown are those equivalent to 'mean+2 SD and mean+3 SD' for data with a Gaussian distribution (n=4995).